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**WO 01/43773 A1**

(54) Title: TNF-ALPHA ANTAGONIST AND LFA-1 ANTAGONIST FOR TREATING LFA-1 OR TNF-ALPHA MEDIATED DISORDER

(57) Abstract: The present invention relates to a method for the treating of an LFA-1 mediated disorder or a TNF- $\alpha$  mediated disorder by administering effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist.

TNF-ALPHA ANTAGONIST AND LFA-1 ANTAGONIST FOR TREATING LFA-1 OR TNF-ALPHA MEDIATED DISORDER

## Field of the Invention

The present invention relates generally to a method for the treating a lymphocyte function associated (LFA)-1 mediated disorder or a tumor necrosis factor (TNF)- $\alpha$  mediated disorder by administering effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist. The invention also relates to treatment of arthritis and psoriasis with an LFA-1 antagonist and a TNF- $\alpha$  antagonist.

## Background of the Invention

TNF is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. It plays an important role in the inflammatory processes of rheumatoid arthritis (RA), polyarticular-course juvenile rheumatoid arthritis (JRA), and the resulting joint pathology. Elevated levels of TNF are found in the synovial fluid of RA patients. Two distinct receptors for TNF (TNFRs), a 55 kilodalton protein (p55) and a 75 kilodalton protein (p75), exist naturally as monomeric molecules on cell surfaces and in soluble forms. Biological activity of TNF is dependent upon binding to either cell surface TNFR. The p55 receptor (also termed TNF-R55, TNF-RI, or TNFR.beta) is a 55 kd glycoprotein shown to transduce signals resulting in cytotoxic, anti-viral, and proliferative activities of TNF- $\alpha$ . The p75 receptor (also termed TNF-R75, TNF-RII, or TNFR- $\alpha$ ) is a 75 kDa glycoprotein that has also been shown to transduce cytotoxic and proliferative signals as well as signals resulting in the secretion of GM-CSF.

Monocytes and macrophages secrete cytokines known as tumor necrosis factor-alpha (TNF- $\alpha$ ) and tumor necrosis factor-beta (TNF- $\beta$ ; lymphotoxin) in response to endotoxin or other stimuli. TNF- $\alpha$  is a soluble homotrimer of 17 kD protein subunits (Smith, et al., J. Biol. Chem. 262:6951-6954 (1987)). A membrane-bound 26 kD precursor form of TNF also exists (Kriegler, et al., Cell 53:45-53 (1988)). For reviews of TNF, see Beutler, et al., Nature 320:584 (1986), Old, Science 230:630 (1986), and Le, et al., Lab. Invest. 56:234. Cells other than monocytes or macrophages also make TNF- $\alpha$ . For example, human non-monocytic tumor cell lines produce TNF (Rubin, et al., J. Exp. Med. 164:1350 (1986); Spriggs, et al., Proc. Natl. Acad. Sci. USA 84:6563 (1987)). CD4<sup>+</sup> and CD8<sup>+</sup> peripheral blood T lymphocytes and some cultured T and B cell lines (Cuturi, et al., J. Exp. Med. 165:1581 (1987); Sung, et al., J. Exp. Med. 168:1539 (1988)) also produce TNF- $\alpha$ .

TNF causes pro-inflammatory actions which result in tissue injury, such as inducing procoagulant activity on vascular endothelial cells (Pober. et al., J. Immunol. 136:1680 (1986)), increasing the adherence of neutrophils and lymphocytes (Pober. et al., J. Immunol. 138:3319 (1987)), and stimulating the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi. et al., J. Exp. Med. 166:1390 (1987)). TNF is also associated with infections (Cerami. et al., Immunol. Today 9:28 (1988)), immune disorders, neoplastic pathologies (Oliff. et al., Cell 50:555 (1987)), autoimmune pathologies and graft-versus host pathologies (Piguet, et al., J. Exp. Med. 166:1280 (1987)).

TNF also plays a central role in gram-negative sepsis and endotoxic shock (Michie, et al., Br. J. Surg. 76:670-671 (1989); Debets, et al., Second Vienna Shock Forum, p.463-466 (1989); Simpson, et al., Crit. Care Clin. 5:27-47 (1989); Waage, et al., Lancet 1:355-357 (1987); Hammerle, et al., Second Vienna Shock Forum p.

715-718 (1989); Debets, et al., Crit. Care Med. 17:489-497 (1989); Calandra, et al., J. Infect. Dis. 161:982-987 (1990); Revhaug, et al., Arch. Surg. 123:162-170 (1988)), including fever, malaise, anorexia, and cachexia.

Polyclonal murine antibodies to TNF are disclosed by Cerami et al. (EPO Patent Publication 0212489, Mar. 4, 1987). Such antibodies were said to be useful in diagnostic immunoassays and in therapy of shock in bacterial infections. Rubin et al. (EPO Patent Publication 0218868, Apr. 22, 1987) discloses murine monoclonal antibodies to human TNF, the hybridomas secreting such antibodies, methods of producing such murine antibodies, and the use of such murine antibodies in immunoassay of TNF.

Yone et al. (EPO Patent Publication 0288088, Oct. 26, 1988) disclose anti-TNF murine antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. The body fluids of patients with Kawasaki's pathology (infantile acute febrile mucocutaneous lymph node syndrome; Kawasaki, Allergy 16:178 (1967); Kawasaki, Shonika (Pediatrics) 26:935 (1985)) were said to contain elevated TNF levels which were related to progress of the pathology (Yone et al., infra).

Other investigators have described rodent or murine mAbs specific for recombinant human TNF which had neutralizing activity in vitro (Liang, et al., (Biochem. Biophys. Res. Comm. 137:847-854 (1986); Meager, et al., Hybridoma 6:305-311 (1987); Fendly et al., Hybridoma 6:359-369 (1987); Bringman, et al., Hybridoma 6:489-507 (1987); Hirai, et al., J. Immunol. Meth. 96:57-62 (1987); Moller, et al., (Cytokine 2:162-169 (1990)). Some of these mAbs were used to map epitopes of human TNF and develop enzyme immunoassays (Fendly et al., infra; Hirai et al., infra; Moller et al., infra) and to assist in the purification of recombinant TNF (Bringman et al., infra). However, these studies do not provide a basis for producing TNF neutralizing antibodies that can be used for in vivo diagnostic or therapeutic uses in humans, due to immunogenicity, lack of specificity and/or pharmaceutical suitability.

Neutralizing antisera or mAbs to TNF have been shown in mammals other than man to abrogate adverse physiological changes and prevent death after lethal challenge in experimental endotoxemia and bacteremia. This effect has been demonstrated, e.g., in rodent lethality assays and in primate pathology model systems (Mathison, et al., J. Clin. Invest. 81:1925-1937 (1988); Beutler, et al., Science 229:869-871 (1985); Tracey, et al., Nature 330:662-664 (1987); Shimamoto, et al., Immunol. Lett. 17:311-318 (1988); Silva, et al., J. Infect. Dis. 162:421-427 (1990); Opal, et al., J. Infect. Dis. 161:1148-1152 (1990); Hinshaw, et al., Circ. Shock 30:279-292 (1990)).

Putative receptor binding loci of hTNF has been disclosed by Eck and Sprang (J. Biol. Chem. 264(29), 17595-17605 (1989), who identified the receptor binding loci of TNF- $\alpha$  as consisting of amino acids 11-13, 37-42, 49-57 and 155-157.

PCT publication W091/02078 (1991) discloses TNF ligands which can bind to monoclonal antibodies having certain epitopes.

To date, experience with anti-TNF murine mAb therapy in humans has been limited. In a phase I study, fourteen patients with severe septic shock were administered a murine anti-TNF mAb in a single dose from 0.4-10 mg/kg (Exley, A. R. et al., Lancet 335:1275-1277 (1990)). However, seven of the fourteen patients developed a human anti-murine antibody response to the treatment, which treatment suffers from the known problems due to immunogenicity from the use of murine heavy and light chain portions of the antibody. Such immunogenicity causes decreased effectiveness of continued administration and can render treatment ineffective, in patients undergoing diagnostic or therapeutic administration of murine anti-TNF antibodies.

Administration of murine TNF mAb to patients suffering from severe graft versus host pathology has also been reported (Herve, et al., Lymphoma Res. 9:591 (1990)).

ENBREL (etanercept) is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. The Fc component of etanercept contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. Etanercept binds specifically to tumor necrosis factor (TNF) and blocks its interaction with cell surface TNF receptors. It inhibits the activity of TNF and has been shown to affect several animal models of inflammation, including murine collagen-induced arthritis. The main treatment for RA has been with methotrexate. Etanercept is currently being used in the treatment of arthritis; the therapy is described in e.g., Moreland et al., 1999, Ann. Intern. Med. 130:478-486.

Aderka, et al., Isrl. J. Med. Sci. 28:126-130 (1992) discloses soluble forms of TNF receptors (sTNF-Rs) which specifically bind TNF and thus can compete with cell surface TNF receptors to bind TNF (Seckinger, et al., J. Exp. Med. 167:1511-1516 (1988); Engelmann, et al., J. Biol. Chem. 264:11974-11980 (1989)). The cloning and expression of human 55 kd TNF receptor and soluble forms of the receptor have been described (Loetscher, et al., Apr. 20, 1990, Cell 61:351-359; Schall et al., Apr. 20, 1990, Cell 61:361-370; Nophar, et al., EMBO J. 9(10):3269-3278 (1990). Engelmann, et al., J. Biol. Chem. 265(3):1531-1536 (1990), discloses TNF-binding proteins. EP 0 433 900 A1 discloses TNF binding protein I (TBP-I), derivatives and analogs thereof, expression of a DNA encoding the entire human type I TNF receptor, or a soluble domain thereof. WO 92/13095 discloses methods for treating tumor necrosis factor mediated diseases by administration of a therapeutically effective amount of a TNF inhibitor selected from a 30 kDa TNF inhibitor and a 40 kDa TNF inhibitor.

EP 0 526 905 discloses multimers of the soluble forms of TNF receptors, which include portions of the hp55 TNF-receptor, produced by either chemical or recombinant methods which are useful for protecting mammals from the deleterious effects of TNF. WO 92/07076 discloses modified human TNF- $\alpha$  receptor which consists of the first three cysteine-rich subdomains but lacks the fourth Cysteine-rich subdomain of the extracellular binding domain of the 55 kDa or 75 kDa TNF receptor for human TNF- $\alpha$ , or an amino acid sequence having a homology of 90% or more with the TNF receptor sequences. EP 0 412 486 A1 discloses antibodies to TNF binding protein I (TBP-I), and fragments thereof, which can be used as diagnostic assays or pharmaceutical agents, either inhibiting or mimicking the effects of TNF on cells. EP 0 398 327 A1 discloses TNF binding protein (TBP) isolated and purified having inhibitory activity on the cytotoxic effect of TNF, as well as TNF binding protein II and monoclonal antibodies thereto. EP 0 308 378 A2 discloses TNF inhibitory protein and functional derivatives used to antagonize the deleterious effects of TNF.

LFA-1 (consisting of CD11a and CD18 subunits) interaction with ICAM is necessary for T-cell killing, T-helper and B-cell responses, natural killing, and antibody-dependent cytotoxicity. In addition, LFA-1/ICAM interactions are involved in adherence of leukocytes to endothelial cells, fibroblasts, and epithelial cells, facilitating the migration of leukocytes from the vasculature to the sites of inflammation (Collins, T., 1995, Science and Medicine, 28-37; Dustin, ML, et al., 1991, Annual Rev Immunology, 9:27-66).

Using antibodies that interfere with LFA-1/ICAM interactions decreases or inhibits the inflammatory process by blocking the activation of T-cells and/or the extravasation of leukocytes. *In vitro*, monoclonal antibodies against LFA-1 or its ligands have inhibited T-cell activation (Kuypers, T. and Roos, D., 1989.

- Research in Immunology, 140:461-86; Springer, TA. 1987. Annual Rev Immunology, 5:223-52). T-cell dependent B-cell proliferation (Fischer, A. et al., 1986. J Immunol, 136:3198-203), target cell lysis (Krensky, A. et al., 1983, J Immunol, 131:6711-6), and adhesion of T-cells to vascular endothelium (Dustin, ML. et al., 1988. Journal of Cell Biology, 107:321-31). The use of an anti-CD11a antibody to treat psoriasis has been described in WO 0056363. In mice, anti-CD11a antibodies have induced tolerance to protein antigens (Benjamin, R. et al. 1988, European Journal of Immunology, 18:1079-88; Tanaka, Y. et al., 1995, European Journal of Immunology, 25:1555-8). delayed the onset and reduced the severity of experimental autoimmune encephalomyelitis (Gordon, EJ et al., 1995, Journal of Neuroimmunology, 62:153-60), inhibited lupus-associated autoantibody production, and prolonged survival of several types of tissue grafts (Cavazzana-Calco MS, Sarnacki S, Haddad E, et al., Transplantation 1995;59(11):1576-82; Nakakura EK, McCabe SM, Zheng B, Shorthouse RA, et al., Transplantation 1993;55(2):412-7; Connolly MK, Kitchens EA, Chan B, et al, Clinical Immunology and Immunopathology 1994;72(2):198-203; He Y, Mellon J, Apte R, Niederkorn J., Investigative Ophthalmology and Visual Science 1994;35(8):3218-25; Isobe M, Yagita H, Okumura K, Ihara A., Science 1992;255:1125-7; Kato Y, Yamataka A, Yagita H, et al., Ann Surg 1996;223(1):94-100; Nishihara M, Gotoh M, Fukuzaki T, et al., Transplantation Proceedings 1995;27(1):372; Talento A, Nguyen M, Blake T, et al, Transplantation 1993;55(2):418-22; van Dijken PJ, Ghayur T, Mauch P, et al., Transplantation 1990;49(5):882-6). In human clinical studies, murine anti-CD11a monoclonal antibodies have been shown to help prevent graft failure following bone marrow transplantation (Cavazzana-Calco MS, Bordigoni P, Michel G, et al., British Journal of Haematology 1996;93:131-8; Fischer A, Friedrich W, Fasth A., Blood 1991;77(2):249-56; Stoppa AM, Maraninchi D, Blaise D, Viens P, *et al.*, Transplant International 1991;4:3-7) and renal transplantation (Hourmant M, Le Mauff B, Le Meur Y, *et al.*, Transplantation 1994;58(3):377-80; Hourmant M, Bedrossian J, Durand D, *et al.*, Transplantation 1996;62(11):1565-70; Le Mauff B, Hourmant M, Rougier JP, *et al.*, Transplantation 1991;52(2):291-6).

- In rheumatoid arthritis, the main presenting symptoms are pain, stiffness, swelling, and loss of function (Bennett J C. The etiology of rheumatoid arthritis. In Textbook of Rheumatology (Kelley W N, Harris E D, Ruddy S, Sledge C B, eds.) W B Saunders, Philadelphia pp 879-886. 1985). The multitude of drugs used in controlling such symptoms seems largely to reflect the fact that none is ideal. None of the treatments clearly stop progression of joint destruction (Harris E D. Rheumatoid Arthritis: The clinical spectrum. In Textbook of Rheumatology (Kelley, et al., eds.) W B Saunders, Philadelphia pp 915-990, 1985).

- TNF- $\alpha$  is of major importance in the pathogenesis of rheumatoid arthritis. TNF- $\alpha$  is present in rheumatoid arthritis joint tissues and synovial fluid at the protein and mRNA level (Buchan G, et al., Clin. Exp. Immunol 73: 449-455, 1988), indicating local synthesis.

- The normal functional capacity of the joint is diminished in OA or rheumatoid arthritis. The suboptimal functional capacity of the joint cartilage in OA or RA thus predisposes the joint to damage insult including the normal level of mechanical or physical insult applied to the joint during activity. The joint cartilage in OA or RA is also less optimally able to undergo normal repair when damaged. Damage to the joint in OA and RA is thus often progressive and damaged hyaline joint cartilage can be replaced by sub-optimal fibrocartilage. Fibrocartilage has significant physical and biochemical differences than that of normal hyaline or articular cartilage in a normal joint and does not optimally have the same functional capacity.

The degradation associated with osteoarthritis usually initially appears as fraying and fibrillation of the surface. Loss of proteoglycan from the matrix also occurs. As the surface fibrillation progresses, the defects penetrate deeper into the cartilage and cartilage is lost. The subchondral bone thickens, is slowly exposed, and may appear polished. Bony nodules or osteophytes also often form at the periphery of the cartilage surface and occasionally grow over the adjacent eroded areas. If the surface of these bony outgrowths is permeated, vacular outgrowth may occur and cause the formation of tissue plugs containing fibrocartilage.

The use of peptide growth factors has also been examined to promote repair of damaged cartilage. Peptide growth factors are very significant regulators of cartilage growth and cell behavior (*i.e.*, differentiation, migration, division, or matrix synthesis or breakdown) [F. S. *Chen et al.*, *Am J. Orthop.* 26: 396-406 (1997)].

Growth factors that have been previously proposed to stimulate cartilage repair include insulin-like growth factor (IGF-1), [Osborn, *J. Orthop. Res.* 7: 35-42 (1989); Florini & Roberts, *J. Gerontol.* 35: 23-30 (1980)]; basic fibroblast growth factor (bFGF), [Toolan *et al.*, *J. Biomec. Mat. Res.* 41: 244-50 (1998); Sah *et al.*, *Arch. Biochem. Biophys.* 308: 137-47 (1994)]; bone morphogenetic protein (BMP) [Sato & Urist, *Clin. Orthop. Relat. Res.* 183: 180-87 (1984); Chin *et al.*, *Arthritis Rheum.* 34: 314-24 (1991) and transforming growth factor beta (TGF- $\beta$ ) [Hill & Logan, *Prog. Growth Fac. Res.* 4: 45-68 (1992); Guerne *et al.*, *J. Cell Physiol.* 158: 476-84 (1994); Van der Kraan *et al.*, *Ann. Rheum. Dis.* 51: 643-47 (1992)]. Insulin has further been proposed to increase cartilage synthesis, insofar as cultured osteoarthritic cartilage explants treated with insulin and tritiated thymidine and [ $^{35}$ S]-sulfate showed incorporation of the latter in a general synthetic response. J. Posever *et al.*, *J. Orthopaedic Res.* 13: 832-827 (1995). Other methods of stimulating cartilage repair include the antagonisation of molecules which are associated with or aggravate cartilage destruction and use, for example, IL-1 $\alpha$  and nitric oxide.

The great majority of people with RA have a genetic susceptibility associated with increased activation of class II major histocompatibility complex molecules on monocytes and macrophages. The genetic predisposition to RA is further supported by the prevalence of the highly conserved leukocyte antigen DR subtype Dw4, Dw14 and Dw15 in human patients with very severe disease. The activated monocytes and macrophages, in interacting with the appropriate T cells stimulate a cascade of immune events which results in further activation of more monocytes and macrophages, T cells, B cells and endothelial cells. This activation increases the synthesis of adhesion molecules, resulting in attracting even more mononuclear cells and polymorphonuclear cells to the inflamed joint. This influx further results in the secretion of additional chemotactic cytokines, causing the invasion of even more inflammatory cells to the synovium and synovial fluid surrounding the joint.

It is generally believed, that many different arthriogenic stimuli activate the immune response in the immunogenetically susceptible host in RA. Both exogenous infectious agents (Ebstein-Barr Virus, Rubella virus, Cytomegalovirus, Herpes Virus, Human T-cell Lymphotropic Virus, Mycoplasma, and others) and endogenous proteins (collagen, proteoglycans, altered immunoglobulins) have been implicated as the causative agent which triggers an inappropriate host immune response. The end result is the production of an excessive inappropriate immune response directed against the host tissues (*e.g.*, antibodies directed against Type II collagen, antibodies directed against the Fc position of autologous IgG (called "Rheumatoid Factor")). This further amplifies the immune response cartilage destruction are responsible for the progression of rheumatoid arthritis. In rheumatoid arthritis, the main presenting symptoms are pain, stiffness, swelling, and loss of function (Bennett J C. The

etiology of rheumatoid arthritis. In Textbook of Rheumatology (Kelley W N, Harris E D, Ruddy S, Sledge C B. eds.) W B Saunders, Philadelphia pp 879-886. 1985).

The cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-4, IL-8, IL-10, TNF- $\alpha$ , PDGF, FGF, GM-CSF, IFN- $\gamma$ , TGF- $\beta$ . IL-2 and IL-6 enhances the activity of fibroblast-like cells in the synovium. chondrocytes and macrophages. thereby releasing increased amounts of proteoglycans. neutral proteinases such as collagenases, transin and stromelysin. These factors cause the recruitment of osteoclast precursors. ultimately culminating in the destruction of bone and cartilage by the invading proliferative synovium. The destructive cascade is characterized physically by increased thinning of the cartilage layer, decreased proteoglycan synthesis, and diminished load-bearing capacity.

Several combination therapies have been described for treating RA. The combination of etanercept (TNFR:Fc fusion protein) and methotrexate (MTX) was used to treat persistently active RA and found to provide greater clinical benefit than methotrexate alone (Weinblatt et al., Jan. 28. 1999. NEJM 340 (4): 253-259). In another clinical trial, the anti-TNF- $\alpha$  chimeric mouse-human antibody, cA2 (infliximab: Remicade,) was given in combination with low-dose methotrexate to RA patients (Mani et al. 1998. Arthritis & Rheumatism 41(9): 1552-1563). Anti-CD4 mAb was found to prevent collagen-induced arthritis if administered before the onset of clinical disease in the CIA mouse model but was ineffective in treating established disease. Co-administration of anti-CD4 antibody with anti-TNF  $\alpha/\beta$  antibody caused significantly greater reduction in paw swelling and joint erosion than that observed by optimal anti-TNF alone (Williams et al. 1994, PNAS 91: 2762-2766). For other references on combination therapies see Kremer (1998). Arthritis & Rheumatism 41: 1548-1551 and Williams (1998). Springer Semin. Immunopathol. 20:165-180.

Administration of many therapeutic agents rapidly induces adverse side effects, or events, including but not limited to fever, headache, nausea, vomiting, breathing difficulties and changes in blood pressure. These adverse events limit the amount of a drug or therapeutic compound that can be given, which in turn limits the therapeutic effectiveness that could be achieved with higher doses of the drug. Adverse events have also been associated with the initial administration of monoclonal antibodies directed to other cell surface molecules. A humanized anti-CD4 monoclonal antibody induced fever, chills, hypotension and chest tightness when given intravenously to psoriasis and rheumatoid arthritis patients (Isaacs. et al., 1997 Clin Exp Immunol. 110. 158-166). This treatment down-modulated expression of CD4 and caused a reduction in the number of circulating CD4-positive T cells.

In view of the above discussion, there exists a strong need for an effective therapy for the treatment and repair of cartilage, including cartilage damaged as a result of injury and/or disease. There is also a continuing need to develop treatment methods that achieve therapeutic efficacy while minimizing toxicity and adverse events (AE). The present invention fulfills these needs and provides additional advantages that will be apparent from the detailed description below.

#### Summary of the Invention

The invention relates to the treatment of a TNF- $\alpha$  mediated disorder and/or an LFA-1 mediated disorder by administering to a mammal in need thereof effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist.

In one embodiment of the above method, TNF- $\alpha$  mediated disorder and/or an LFA-1 mediated disorder is a joint disorder.

In particular embodiments of the above treatment methods, the LFA-1 mediated disorder or TNF- $\alpha$  mediated disorder is rheumatoid arthritis, juvenile chronic arthritis/early RA, psoriasis, graft rejection (HvGD),  
5 graft versus host disease (GvHD), or multiple sclerosis.

The present invention also concerns methods for the treatment, repair and protection of cartilage, including cartilage damage as a result of degenerative cartilagenous disorders and/or injury. More specifically, the invention concerns method for the treatment, repair and protection of articular cartilage comprising administering effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist. In a further embodiment, the  
10 present invention concerns a method for the treatment of cartilage damaged as a result of a degenerative cartilagenous disorder comprising contacting said cartilage with an effective amount of an LFA-1 antagonist and a TNF- $\alpha$  antagonist. Optionally, the cartilage is articular cartilage, and is contained within a mammal and the effective amount administered to the patient in need thereof is a therapeutically effective amount. Optionally, the degenerative cartilagenous disorder is osteoarthritis or rheumatoid arthritis.

In a further embodiment, the present invention concerns a method for the treatment of cartilage  
15 damaged by injury comprising contacting said cartilage with an effective amount of an LFA-1 antagonist and a TNF- $\alpha$  antagonist. More specifically, the injury treated is a microdamage or blunt trauma, a chondral fracture, or an osteochondral fracture. More specifically, the cartilage is contained within a mammal, including humans, and the amount administered is a therapeutically effective amount.

In a further embodiment, the present invention concerns a method for the treatment of damaged  
20 cartilage or for preventing initial or continued damage of cartilage as a result of a degenerative cartilagenous disorder and/or injury comprising contacting said cartilage with an effective amount of a composition comprising an LFA-1 antagonist and a TNF- $\alpha$  antagonist. The composition may further comprise a carrier, excipient or stabilizer. Alternatively, the cartilage is present in a mammal and the amount administered is a therapeutically  
25 effective amount. The composition may be administered via injection or infusion by intravenous, intraarterial, intraperitoneal, intramuscular, intralesional, intraarticular or topical administration to a mammal and the amount administered is a therapeutically effective amount. Alternatively, the composition is injected directly into the afflicted cartilagenous region or joint.

In a further embodiment, the present invention concerns a method for the treatment of cartilage damage  
30 or preventing initial or continued damage of cartilage as a result of a degenerative cartilagenous disorder and/or injury comprising administering a therapeutically effective amount of an extended-release composition containing an LFA-1 antagonist and a TNF- $\alpha$  antagonist. Preferably, the cartilage is present in a mammal and the amount administered is a therapeutically effective amount. More specifically, the extended-release composition contain an LFA-1 antagonist and a TNF- $\alpha$  antagonist formulated in a microencapsulation, a semi-  
35 permeable membrane of solid hydrophobic polymers, a biodegradable polymer(s), or a dispersion (*e.g.*, suspension or emulsion). More specifically, the semi-permeable membrane of solid hydrophobic polymer is poly-lactic-co-glycolic acid (PLGA), and the biodegradable polymer is cross-linked hyaluronic acid (HA). Alternatively, the extended-release composition further comprises a water-soluble polyvalent metal salt. More specifically, the polyvalent metal salt includes the salt formed from an alkaline earth metal and an inorganic or  
40 organic acid.



In a further embodiment, the invention concerns a method for treating cartilage damaged or preventing initial or continued damage as a result of injury or a degenerative cartilaginous disorder comprising contacting the cartilage with effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist in combination with an effective amount of cartilage growth factor. Optionally, the cartilage is present inside a mammal and the amount administered is a therapeutically effective amount. More specifically, the cartilage growth factor may be insulin-like growth factors (*e.g.*, IGF-1, IGF-2), platelet-derived growth factor (PDGF), bone morphogenic proteins (BMPs), disruptors or down regulators of *c-myc* or *Bcl-2* expression, antisense RNA or DNA or disruption of associated promoter regions. Optionally the cartilage growth factor may be an agent which enhances the reparative response of intrinsic cartilage, such as through increasing the actual or potential proliferation of chondrocytes (*e.g.*, basic fibroblast growth factor (bFGF)), or through the forced progression of cell differentiation cell cycle progression factors such as IGF's, TGF- $\beta$  and epidermal growth factors (EGF). Optionally, the cartilage growth factor may be an agent which antagonizes the catabolism of cartilage (*e.g.*, IL-1 receptor antagonist (IL-1ra), NO inhibitors).

In a further embodiment, the present invention concerns a method of treating cartilage damaged or preventing initial or continued damage of cartilage comprising contacting said cartilage with an effective amount of an LFA-1 antagonist and a TNF- $\alpha$  antagonist in combination with an effective amount of a cartilage catabolism antagonist. Optionally, the cartilage is articular cartilage, and is contained within a mammal and the amount administered of each agent is a therapeutically effective amount.

In a further embodiment, the present invention concerns a method for the treatment of cartilage damaged by injury comprising contacting said cartilage with an effective amount of an LFA-1 antagonist and a TNF- $\alpha$  antagonist in combination with a cartilage catabolism antagonist. More specifically, the injury treated is a microdamage or blunt trauma, a chondral fracture, or an osteochondral fracture. More specifically, the cartilage is contained within a mammal, including humans, and the amount administered of each agent is a therapeutically effective amount.

Yet another embodiment of the invention is a method of preventing the development or delaying the onset of rheumatoid arthritis in subjects genetically disposed or susceptible to developing rheumatoid arthritis by administering to the subject, an effective amount of an LFA-1 antagonist and a TNF- $\alpha$  antagonist. In a specific embodiment, the RA is juvenile RA and the subject is a juvenile (under age 16).

LFA-1 antagonism may allow the use of lower doses of drugs for TNF- $\alpha$  antagonism to attain the same or better efficacy but with reduced clinical adverse events. Thus, a further aspect of the invention is a method of reducing adverse events associated with the administration of an LFA-1 antagonist by reducing the dose of the LFA-1 antagonist to a suboptimal or subtherapeutic dose (*i.e.*, lower than the recommended therapeutically effective dose) but administering a TNF- $\alpha$  antagonist in combination. This method may be advantageous in the treatment of pediatric patients. Thus, the method of reducing adverse events associated with the administration of an LFA-1 antagonist involves the administration of a TNF- $\alpha$  antagonist, and an LFA-1 antagonist at a subtherapeutic dose. This method can also be applied to reducing adverse events associated with the administration of a TNF- $\alpha$  antagonist by administering the TNF- $\alpha$  antagonist with an LFA-1 antagonist wherein the TNF- $\alpha$  antagonist is administered at a subtherapeutic dose. In a specific embodiment, the LFA-1 antagonist is anti-CD11a antibody and the TNF- $\alpha$  antagonist is etanercept. The therapeutic/optimal doses for anti-CD11a antibody hu1124 and for etanercept are available from the drug product literature.

Yet another aspect is a method of treating rheumatoid arthritis is by administering to a patient in need thereof effective amounts of an LFA-1 antagonist, a TNF- $\alpha$  antagonist and methotrexate.

In a preferred embodiment of all the treatment methods of the invention, the LFA-1 antagonist is an anti-CD11a antibody. It is further preferred that the anti-CD11a antibody be a non-lymphocyte depleting, in particular, non-T cell depleting antibody. The anti-CD11a antibody, hu1124, is non-T cell depleting. In more specific embodiments, anti-CD11a antibody is a human or humanized antibody or antibody fragment thereof, most preferably, the humanized antibody hu1124 disclosed and claimed in U.S. patent 6,037,454. In a preferred embodiment of all the treatment methods of the invention, the TNF- $\alpha$  antagonist is an immunoadhesin, preferably a fusion of at least a portion of a TNF- $\alpha$  binding protein and a portion of an immunoglobulin, more preferably a TNF- $\alpha$  receptor - IgG Fc fusion protein such as etanercept.

In a specific embodiment of all the above methods of the invention, the joint or cartilage disorder is rheumatoid arthritis.

In another embodiment, the present invention concerns a therapeutic kit, comprising an LFA-1 antagonist and a TNF- $\alpha$  antagonist and a carrier, excipient and/or stabilizer (*e.g.* a buffer) in suitable packaging. The kit preferably contains instructions for using an LFA-1 antagonist and a TNF- $\alpha$  antagonist to treat an LFA-1 or a TNF- $\alpha$  mediated disorder. Alternatively, the kit may contain instructions for using an LFA-1 antagonist and a TNF- $\alpha$  antagonist to treat a degenerative cartilagenous disorder, such as rheumatoid arthritis.

In a further embodiment, the invention concerns an article of manufacture, comprising:  
a container;  
an instruction on the container; and  
a composition comprising an active agent contained within the container;  
wherein the composition is effective for treating a degenerative cartilagenous disorder, the instruction on the container indicates that the composition can be used to treat an LFA-1 or a TNF- $\alpha$  mediated disorder. In a preferred aspect, the active agent is an LFA-1 antagonist and a TNF- $\alpha$  antagonist.

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#### Brief Description of the Drawing

Figure 1 shows the effect of treatment with a combination of an LFA-1 antagonist and a TNF antagonist in reducing the incidence of clinical arthritis in animals (see Example 1).

Figure 2 shows the effect of treatment with either anti-murine CD11a antibody (M17) alone, or TNF antagonist (Enbrel) alone, on arthritis in DBA-1LacJ mice, as indicated by the mean clinical scores (see Example 2). Saline treatment served as a control.

Figure 3 shows the effect of treatment with anti-murine CD11a antibody (M17) alone, or saline (control), on arthritis in DBA-1J mice, as indicated by the mean clinical scores (see Example 2).

Figure 4 shows the effectiveness of treatment with a combination of an LFA-1 antagonist (antibody M17) and a TNF antagonist (Enbrel), as compared to the individual antagonist alone, in reducing clinical arthritis in DBA-1LacJ mice (see Example 3).

Figure 5 shows the effectiveness of treatment with a combination of an LFA-1 antagonist (antibody M17) and a TNF antagonist (Enbrel), as compared to the individual antagonist alone, in reducing clinical arthritis in DBA-1J mice (see Example 3).

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Detailed Description of the Preferred EmbodimentsI. Definitions

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

The term "antagonist" with respect to LFA-1 or TNF- $\alpha$  means a compound which is capable of, directly or indirectly, counteracting, reducing or inhibiting the biological activity of LFA-1 or TNF- $\alpha$  or activation of receptors therefor.

"ENBREL" (etanercept) is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. The Fc component of etanercept contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. Etanercept binds specifically to tumor necrosis factor (TNF) and blocks its interaction with cell surface TNF receptors. It inhibits the activity of TNF.

"Biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring molecule, such as LFA-1 or TNF- $\alpha$ , other than the ability to serve as an antigen in the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring polypeptide of the invention. Similarly, an "immunological" activity refers to the ability to serve as an antigen in the production of an antibody against an antigenic epitope possessed by the antigen. Some of the biological activities of TNF- $\alpha$  and LFA-1 are described in the background and throughout the specification.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONIC<sup>TM</sup>.

"Cartilage growth factor" as used herein, refers to agent(s) other than an LFA-1 antagonist or a TNF antagonists which cause, induce or result in an improvement in the condition of or protection from initial or continued destruction of cartilage subject to damage by either by injury or a degenerative cartilagenous disorder. Such cartilage growth factors include insulin-like growth factors (*e.g.*, IGF-1, IGF-2), platelet-derived growth factor (PDGF), bone morphogenic proteins (BMPs), disruptors or down regulators of *c-myc* or *Bcl-2* expression, antisense RNA or DNA or disruption of associated promotor regions. Optionally the cartilage growth factor may be an agent which enhances the reparative response of intrinsic cartilage, such as through increasing the actual or potential proliferation of chondrocytes (*e.g.*, basic fibroblast growth factor (bFGF)), or through the forced progression of cell differentiation cell cycle progression factors such as IGF's, TGF- $\beta$  and epidermal growth factors (EGF).

A further subset of molecules which fall under the above definition of "cartilage growth factor" are agents which antagonize the catabolism of cartilage or a "cartilage catabolism antagonist." Cartilage catabolism

antagonists may be defined as those agents which inhibit, attenuate or otherwise block the activity or effect of molecules that are associated with or aggravate cartilage destruction. For example, IL-1 $\alpha$  and nitric oxide (NO) are agents known to be associated with cartilage destruction. Thus, inhibitors of IL-1 $\alpha$  (e.g., IL-1ra) and NO production would be considered "cartilage catabolism antagonists. Moreover, antagonists of chondrocyte catabolism (e.g., sodium pentosan polysulfate) would also be considered cartilage catabolism antagonists.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time.

"Intermittent" administration is treatment that is done not consecutively without interruption, but rather is cyclic in nature.

A "conditioning dose" is a dose which attenuates or reduces the frequency or the severity of first dose adverse side effects associated with administration of a therapeutic compound. The conditioning dose may be a therapeutic dose, a sub-therapeutic dose, a symptomatic dose or a sub-symptomatic dose. A therapeutic dose is a dose which exhibits a therapeutic effect on the patient and a sub-therapeutic dose is a dose which does not exhibit a therapeutic effect on the patient treated. A symptomatic dose is a dose which induces at least one adverse effect on administration and a sub-symptomatic dose is a dose which does not induce an adverse effect.

The term "an LFA-1 and/or TNF- $\alpha$  mediated disorder" refers to pathological states caused by either LFA-1 cell adherence interactions on lymphocytes or TNF- $\alpha$  binding interactions with a TNF receptor or both. Some disorders may involve both LFA-1 cell adherence interactions and TNF- $\alpha$  binding interactions and therefore may be an LFA-1 mediated as well as a TNF- $\alpha$  mediated disorder.

Included within the scope of "articular cartilage disorder" is osteoarthritis (OA) and rheumatoid arthritis (RA). OA defines not a single disorder, but the final common pathway of joint destruction resulting from multiple processes. OA is characterized by localized asymmetric destruction of the cartilage commensurate with palpable bone enlargements at the joint margins. OA typically affects the interphalangeal joints of the hands, the first carpometacarpal joint, the hips, the knees, the spine, and some joints in the midfoot, while large joints, such as the ankles, elbows and shoulders tend to be spared. OA is sometimes also associated with metabolic diseases such as hemochromatosis and alkaptonuria, developmental abnormalities such as developmental dysplasia of the hips (congenital dislocation of the hips), limb-length discrepancies, including trauma and inflammatory arthritides such as gout, septic arthritis, and neuropathic arthritis.

The term "degenerative cartilagenous disorder" refers to a collection of diseases which are characterized, at least in part, by degeneration or metabolic derangement of the connective tissue structures of the body, especially the joints of related structures, including muscles, bursae (synovial membrane), tendons and fibrous tissue. These diseases are further manifested by the symptoms of pain, stiffness and/or limitation of motion of the affected body parts. In one embodiment, the term includes "articular cartilage disorders" which are characterized by disruption of the smooth articular cartilage surface and degradation of the cartilage matrix. Additional pathologies include nitric oxide production, and elevated proteoglycan breakdown.

Furthermore, the term "degenerative cartilagenous disorder" may include systemic lupus erythematosus and gout, amyloidosis or Felty's syndrome. Additionally, the term covers the cartilage degradation and destruction associated with psoriatic arthritis, acute inflammation (e.g., yersinia arthritis, pyrophosphate arthritis, gout arthritis (arthritis urica), septic arthritis), arthritis associated with trauma, inflammatory bowel disease (e.g., ulcerative colitis, Crohn's disease, regional enteritis, distal ileitis, granulomatous enteritis, regional ileitis.

terminal ileitis), multiple sclerosis, diabetes (*e.g.*, insulin-dependent and non-insulin dependent), obesity, giant cell arthritis and Sjögren's syndrome. Examples of other immune and inflammatory diseases which may be treated by the method of the invention include juvenile chronic arthritis and spondyloarthropathies.

Rheumatoid arthritis (RA) is a systemic, chronic, autoimmune disorder characterized by symmetrical synovitis of the joint and typically affects small and large diarthroid joints alike. As RA progresses, symptoms may include fever, weight loss, thinning of the skin, multiorgan involvement, scleritis, corneal ulcers, the formation of subcutaneous or subperiosteal nodules and even premature death. The symptoms of RA often appear during youth and can include vasculitis, atrophy of the skin and muscle, subcutaneous nodules, lymphadenopathy, splenomegaly, leukopaenia and chronic anaemia.

The term "effective amount" refers to the minimum concentrations of an LFA-1 antagonist and a TNF antagonist which cause, induce or result in either a detectable or measurable improvement or repair in damaged cartilage or a measurable protection from the continued or induced cartilage destruction in an isolated sample of cartilage matrix. For example, the inhibition of release of free proteoglycan from the cartilage tissue.

A "therapeutically effective amount" refers to the minimum concentrations (amount) of an LFA-1 antagonist and a TNF antagonist administered to a mammal that are effective in at least attenuating a pathological symptom (*e.g.* causing, inducing or resulting in a detectable / measurable improvement: lessen the severity, extent or duration of symptoms) which occurs as a result of an LFA-1 and/or a TNF- $\alpha$  mediated disorder. The symptoms will vary with the particular disorder: however, the symptoms of a particular disorder and the means of detecting or measuring improvement in the symptoms will be familiar to the physician of skill in the art. As examples, the symptoms of RA and psoriasis are described below. For example, the therapeutically effective amount is effective in causing, inducing or resulting in either a detectable / measurable improvement or repair in damaged articular cartilage or causing, inducing or resulting in a measurable protection from the continued or initial cartilage destruction, improvement in range of motion, reduction in pain, *etc.*) which occurs as a result of injury or a degenerative cartilagenous disorder.

In treating rheumatoid arthritis (RA) in humans, the criteria for evaluating extent of or improvement in the disease may include for example, assessment of the number of tender and swollen joints, patient and physician global assessment (*e.g.*, at 3 and 6 months from initiation of treatment), morning stiffness, pain, increased functional status (*e.g.*, through a Health Assessment Questionnaire), disability, structural damage, and acute phase reactant. Preferably, the amounts of LFA-1 antagonist and TNF antagonist are effective to achieve in the patient, at least a 20% improvement in at least one of the preceding criteria, more preferably at least 30%, even more preferably, at least 40% or 50%, most preferably at least 75% improvement compared to the control or placebo treated patient. Alternatively, an improvement in at least one grade in clinical scores, *e.g.*, in the Paulus criteria is considered effective treatment herein.

Disability and acute phase reactants are taught in Felson, DT et al. 1993, The American College of Rheumatology Preliminary Core Set of Disease Activity for Rheumatoid Arthritis Clinical Trials. Arthritis and Rheumatism, 36 (6): 729-740; and Felson, DT et al, 1995. The American College of Rheumatology Preliminary Definition of Improvement in Rheumatoid Arthritis. Disease, Arthritis and Rheumatism, 38 (40): 1-9. Structural damage can be evaluated by radiography which can reveal slowing X-ray progression of the disease based on a validated radiographic index such as the Larsen or modified Sharp index. One can also evaluate radiographically to determine if treatment prevents the formation of new joint erosions or slows the progression. Other

methodologies could be employed such as magnetic resonance imaging, ultrasonography, and NMR. The American College of Rheumatology (ACR) response criteria or alternatively, the Paulus criteria are well known criteria used in evaluating drug efficacy in treating rheumatoid arthritis. ACR criteria is based on tender joint count and swollen joint count; (1) patient pain assessment, (2) patient global assessment, (3) physician global assessment, (4) patient self-assessed disability, and (5) acute-phase reactant (ESR or CRP). The Paulus Criteria relies on improvement in at least four of the following: Tender joint score; Swollen joint score; Morning stiffness; Patient assessment of disease severity (5 point scale); Physician assessment of disease severity (5 point scale); and ESR.

Psoriasis is another LFA-1 and TNF- $\alpha$  mediated disorder. Efficacy of psoriasis treatment can be monitored by changes in clinical signs and symptoms of the disease, including Psoriasis Area and Severity Index (PASI) scores, physician's global assessment (PGA) of the patient compared with the baseline condition. A decrease in PASI score indicates a therapeutic effect. Psoriatic disease activity can also be determined based on Overall Lesion Severity (OLS) scale, percentage of total body surface area (BSA) affected by psoriasis, and psoriasis plaque thickness. Skin biopsies are studied for the effects of the drug on lymphocytes within psoriatic lesions. Histological analysis of skin biopsies can be performed to look for reduction in epidermal thickness and T-cell infiltration and reversal of pathological epidermal hyperplasia. Immunological activity can be monitored by testing for the effects of treatment on cell-mediated immunity reactions (delayed hypersensitivity), tetanus antibody responses, and lymphocyte subpopulations (flow cytometry).

For asthma, one indicator of therapeutic effect is a decrease in nonspecific airway hyperresponsiveness to methacholine challenges (basal and post-allergen), upon treatment by the method of the invention. Airway hyperresponsiveness can be measured by FEV<sub>1</sub> (volume of air that can be forced from the lungs in 1 second).

For transplant or graft survival and function, therapeutic effectiveness can be measured, *e.g.*, by the incidence of acute graft rejection, by graft function, and length of graft survival.

The term "extended-release" or "sustained-release" formulations in the broadest possible sense means a formulation of active an LFA-1 antagonist or a TNF antagonist polypeptide resulting in the release or activation of the active polypeptide for a sustained or extended period of time - or at least for a period of time which is longer than if the polypeptide was made available *in vivo* in the native or unformulated state. Optionally, the extended-release formulation occurs at a constant rate and/or results in sustained and/or continuous concentration of the active polypeptide. Suitable extended release formulations may comprise microencapsulation, semi-permeable matrices of solid hydrophobic polymers, biodegradable polymers, biodegradable hydrogels, suspensions or emulsions (*e.g.*, oil-in-water or water-in-oil). Optionally, the extended-release formulation comprises polylactic-co-glycolic acid (PLGA) and can be prepared as described in Lewis, "Controlled Release of Bioactive Agents from Lactide/Glycolide polymer," in *Biodegradable Polymers as Drug Delivery Systems*, M. Chasin & R. Langer, Ed. (Marcel Dekker, New York), pp. 1-41. Optionally, the extended-release formulation is stable and the activity of the an LFA-1 antagonist or a TNF antagonist does not appreciably diminish with storage over time. More specifically, such stability can be enhanced through the presence of a stabilizing agent such as a water-soluble polyvalent metal salt.

The term "immunoadhesin" refers to a chimeric molecule which is a fusion of a ligand binding moiety, such as a receptor extracellular domain, with an immunoglobulin or a particular region of an immunoglobulin.

For a bivalent form of an immunoadhesin, such a fusion could be to the Fc region of an IgG molecule. In a

particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130. As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.* is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cattle, *etc.* Preferably, the mammal is human.

The "pathology" of a degenerative cartilagenous disorder includes all physiological phenomena that compromise the well-being of the patient. This includes, without limitation, cartilage destruction, diminished cartilage repair, abnormal or uncontrollable cell growth, antibody production, auto-antibody production, complement production and activation, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of any inflammatory or immunological response, infiltration of inflammatory cells (neutrophilic, eosinophilic, monocytic, lymphocytic) into tissue spaces, *etc.*

A "small molecule" is defined herein to have a molecular weight below about 600 daltons, and is generally an organic compound.

By "solid phase" is meant a non-aqueous matrix to which the compound of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.* controlled pore glass), polysaccharides (*e.g.* agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.* an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, slow down or lessen the severity, extent or duration of symptoms, or delay the onset of (*e.g.* in subjects predisposed to develop RA due to genetic make-up or other risk factors) the targeted pathological condition or disorder. "Treating" a disease, disorder, condition or cell population includes therapy and prophylactic treatment on an acute short term basis and on a chronic long-term basis. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Treatment is successful if it results in a detectable or measurable improvement in at least one

symptom of the disorder the LFA-1 and/or a TNF- $\alpha$  mediated disorder being treated (consistent with the definition of "therapeutically effective amount" above).

In treatment of a degenerative cartilagenous disorder, a therapeutic agent may directly decrease or increase the magnitude of response of a pathological component of the disorder, or render the disease more susceptible to treatment by other therapeutic agents, *e.g.* antibiotics, antifungals, anti-inflammatory agents, chemotherapeutics, *etc.*

## II. Modes for Carrying Out the Invention

### A. Antagonists

Suitable LFA-1 antagonists include any compound which inhibits the interaction of LFA-1 and a receptor therefor, in particular, ICAM-1. The LFA-1 antagonist may be a small molecule, peptide, protein, immunoadhesin, an anti-LFA-1 antibody, or a fragment thereof, for example. These terms refer to antagonists directed against either CD11a or CD18 or both. Anti-CD11a antibodies include, *e.g.*, MHM24 [Hildreth *et al.*, Eur. J. Immunol., **13**: 202-208 (1983)], R3.1 (IgG1) [R. Rothlein, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT], 25-3 (or 25.3), an IgG1 available from Immunotech, France [Olive *et al.*, in Feldmann, ed., Human T cell Clones. A new Approach to Immune Regulation, Clifton, NJ, Humana, 1986 p. 173], KBA (IgG2a) [Nishimura *et al.*, Cell. Immunol., **107**: 32 (1987); Nishimura *et al.*, *ibid.*, **94**: 122 (1985)], M7/15 (IgG2b) [Springer *et al.*, Immunol. Rev., **68**: 171 (1982)], IOT16 [Vermot Desroches *et al.*, Scand. J. Immunol., **33**: 277-286 (1991)], SPVL7 [Vermot Desroches *et al.*, *supra*], and M17 (IgG2a), available from ATCC, which are rat anti-murine CD11a antibodies. Preferred anti-CD11a antibodies are the humanized antibodies described in U.S. Patent 6,037,454. It is also generally preferred that the anti-CD11a antibodies are not T-cell depleting antibodies, that is, that the administration of the anti-CD11a antibody does not reduce the level of circulating T-cells.

Examples of anti-CD18 antibodies include MHM23 [Hildreth *et al.*, *supra*], M18/2 (IgG2a) [Sanches-Madrid *et al.*, J. Exp. Med., **158**: 586 (1983)], H52 [Fekete *et al.*, J. Clin. Lab Immunol., **31**: 145-149 (1990)], Mas191c [Vermot Desroches *et al.*, *supra*], IOT18 [Vermot Desroches *et al.*, *supra*], 60.3 [Taylor *et al.*, Clin. Exp. Immunol., **71**: 324-328 (1988)], and 60.1 [Campana *et al.*, Eur. J. Immunol., **16**: 537-542 (1986)]. See also US 5,997,867.

Other examples of suitable LFA-1 binding molecules, including antibodies, are described in Hutchings *et al.*, *supra*, WO 98/51343, WO 91/18011, WO 91/16928, WO 91/16927, Can. Pat. Appln. 2,008,368, WO 90/15076, WO 90/10652, WO 90/13281, WO 93/06864, WO 93/21953, EP 387,668, EP 379,904, EP 346,078, US 5,932,448, US 5,622,700, US 5,597,567, U.S. 5,071,964, U.S. 5,002,869, US 5,730,983, Australian Pat. Appln. 8815518, FR 2700471A, EP 289,949, EP 362526, and EP 303,692. Preferred LFA-1 binding antibodies for use in the invention are disclosed in U.S. Patent 6,037,454.

Suitable TNF- $\alpha$  antagonists include any compound which inhibits the interaction of TNF- $\alpha$  and a receptor therefor, in particular, the p55 receptor and the p75 receptor. The TNF- $\alpha$  antagonist may be a small molecule, peptide, protein, receptor extracellular domain, immunoadhesin or an anti-TNF- $\alpha$  antibody, for example.

The TNF- $\alpha$  antagonists include ENBREL, etanercept (Immunex/AHP); Remicade®, Infliximab, which is an anti-TNF chimeric Mab (Centocor/Johnson&Johnson); anti-TNF $\alpha$ , D2E7 human Mab (Cambridge Antibody



Technology); CDP-870 which is a PEGylated antibody fragment (Celltech); CDP 571, Humicade, which is a humanized Mab (Celltech); PEGylated soluble TNF- $\alpha$  Receptor I (Amgen); TBP-1 which is a TNF binding protein (Ares Serono); PASSTNF-alpha® which is an anti-TNF- $\alpha$  polyclonal antibody (Verigen); AGT-1 (from Advanced Biotherapy Concepts) which is a mixture of 3 anti-cytokine antibodies, antibodies to IFN- $\alpha$ , IFN- $\gamma$ , and TNF; TENEFUSE, lenercept which is a TNFR-Ig fusion protein (Roche); CytoTab® (Protherics); TACE which is a small molecule TNF- $\alpha$  converting enzyme inhibitor (Immunex); small molecule TNF mRNA synthesis inhibitor (Nereus); PEGylated p75 TNFR Fc mutein (Immunex); and TNF- $\alpha$  antisense inhibitor.

Molecular cloning has demonstrated the existence of two distinct types of TNF receptors (TNFR) with apparent molecular masses of 55kD (type 1) (Schall et al., (1990) Cell 61:361) and 75 kD (type 2) (Smith et al., (1990) Science 248:1019), each of which naturally binds to both TNF- $\alpha$  and TNF- $\beta$  (Loetscher et al., (1990) Cell, 61:351; Shall et al. (1990) Cell, 61:361; Kohno et al., 1990) Proc. Natl. Acad. Sci. USA 87:8331). The extracellular portions of both receptors are found naturally as soluble TNF binding proteins (Kohno et al., supra). TNF antagonists have been created which block the deleterious effect of TNF in various immune and inflammatory events (Peppel et al., (1991) J. Exp. Med., 174:1483-1489; Ulich (1993) Am. J. Path., 142:1335-1338; Howard, O.M.Z., (1993) Proc. Natl. Acad. Sci. USA 90:2335-2339; Wooley, P.H., (1993) J. Immunol. 151:6602-6607). One such antagonist (Werner et al., (1991) J. Cell. Biochem. Abstracts, 20th annual meeting, p. 115) combines the extracellular domain of human 55 kD type 1 TNFR with a portion of the hinge and Fc regions of human immunoglobulin G1 heavy chain. Another such antagonist (Mohler et al., (1993) J. Immunol. 151:1548-1561) combines the extracellular domain of human 75 kD type 2 TNFR with a portion of the hinge and Fc regions of human immunoglobulin G1 heavy chain. U.S. patents 5,482,130 and 5,514,582 describe these molecules. Any of these molecules may be used as the TNF- $\alpha$  antagonist of the invention.

Other examples of TNF- $\alpha$  antagonists include the anti-TNF- $\alpha$  antibodies disclosed in US 5,795,967; WO 97/29131 (which discloses recombinant human antibodies and antibodies produced using phage display techniques); US 5,654,407 and US 5,994,510 (which disclose human anti-TNF- $\alpha$  antibodies); WO 92/11383 and WO 92/16553 (which disclose chimeric, including humanized, antibodies); US 5,656,272, US 5,919,452 and US 5,698,195 (which disclose chimeric antibodies); and Fendley et al. 1987, Hybridoma 6:359 and Bringman et al. 1987, Hybridoma 6:489 (which disclose additional anti-TNF- $\alpha$  antibodies).

Additional examples of suitable TNF- $\alpha$  antagonists include immunoadhesins containing at least a TNF- $\alpha$  binding portion of a TNF- $\alpha$  receptor. Preferred immunoadhesins are disclosed in US 5,605,690 and 5,712,155, for example. Other suitable TNF- $\alpha$  antagonists are described in US 5,482,130; US 5,514,582; US 5,336,603 and US 5,565,335.

Other suitable TNF- $\alpha$  antagonists include compounds which reduce the levels of TNF- $\alpha$  in tissues including the compounds disclosed in US 5,994,510; US 5,985,620; US 5,981,701, US 5,594,106; US 5,629,285 and US 5,945,397.

In another embodiment, the TNF- $\alpha$  antagonist is a TNF- $\alpha$  receptor - IgG Fc fusion protein, such as ENBREX (Immunex) and the LFA-1 antagonist is an anti-CD11a antibody, preferably a non T-cell depleting anti-CD11a antibody such as hu1124 (XOMA/Genentech).

The LFA-1 antagonist and the TNF- $\alpha$  antagonist may be administered in amounts conventionally used for these compounds. The compounds may be administered at a molar ratio of about 1:1000 to about 1000:1, or

about 100:1 to about 1:100, or about 1:10 to about 10:1, or in a ratio of about 1:5 to about 5:1, or even at a ratio of about 1:1.

#### B. Administration

5 The combination of compounds of the present invention are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, intralesional, intraarticular or inhalation (intranasal, intrapulmonary, aerosolized) routes and by sustained release or extended-release means. Optionally the active compound or  
10 formulation is injected directly into an afflicted cartilagenous region or articular joint.

Administration of an LFA-1 antagonist and a TNF antagonist, separately or together, may be in dosage amounts for each compound varying from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature for each of the compounds. For  
15 example, ENBREL is currently recommended at a dosage of 25 mg for adult humans, twice weekly as subcutaneous injection given at least 72-96 hours apart. In one case, up to 62 mg ENBREL has been administered to an adult subcutaneously (SC) twice weekly for 3 weeks without producing adverse effects (see PDR). The recommended dose of ENBREL for pediatric patients ages 4 to 17 years with active polyarticular-course JRA is 0.4 mg/kg (up to a maximum of 25 mg per dose) given twice weekly as a subcutaneous injection  
20 72-96 hours apart. Methotrexate (see Weinblatt et al., Jan. 28, 1999; Mani et al. 1998, supra), glucocorticoids, salicylates, nonsteroidal anti-inflammatory drugs (NSAIDs), or analgesics may be continued during treatment with ENBREL. An LFA-1 antagonist, humanized anti-CD11a antibody hu1124, can be administered at a dosage range of between 0.3 mg/kg to 6 mg/kg. LFA-1 antagonism may allow the use of lower doses of drugs for TNF antagonism, and vice versa, to attain the same or better efficacy but with reduced clinical adverse events  
25 including but not limited to fever, chills, infection, sepsis and anemia. Thus, in the treatment methods of the present invention, dosages of one or both of the antagonists can be reduced to minimize any toxicity or adverse events that can occur with administration of the normal or recommended dose for either antagonist alone. For example, when used together in the present treatment methods, the aforementioned dosages for ENBREL and hu1124 can be reduced, especially in the treatment of juveniles (e.g., for Juvenile RA).

30 The appropriate dosages of the compounds of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the  
35 determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" in *Toxicokinetics and New Drug Development*, Yacobi et al., Eds., Pergamon Press, New York 1989, pp. 42-96.

40 The doses may be administered according to any time schedule which is appropriate for treatment of the disease or condition. For example, the dosages may be administered on a daily, weekly, biweekly or monthly

basis in order to achieve the desired therapeutic effect and reduction in adverse effects. The compound is suitably administered to the patient at one time or over a series of treatments. The dosages can be administered before, during or after the development of the disorder. For example, to prevent host versus graft or graft versus host rejection, the initial dose may be administered before, during or after transplantation has occurred. The specific time schedule can be readily determined by a physician having ordinary skill in administering the therapeutic compound by routine adjustments of the dosing schedule within the method of the present invention.

The dosing schedule may include a first conditioning dose of one or both antagonists followed by a second higher or therapeutic dose of the antagonists, to condition the mammal to tolerate increasing or higher doses of the therapeutic compounds. This dosing schedule allows one to reduce the occurrence of adverse effects which arise from the initial administration and subsequent administrations of the therapeutic compound (see WO 0056363). Although some adverse effects such as fever, headache, nausea, vomiting, breathing difficulties, myalgia, chills and changes in blood pressure may still be observed, the frequency and/or severity of these adverse effects may be reduced relative to administration using conventional dosing schedules such as daily administration of equal doses of a therapeutic compound.

For example, depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (e.g. 0.1-20 $\text{mg/kg}$ ) of each of the compounds of the invention is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  or more, depending on the factors mentioned above. A preferred dose of about 0.1-30  $\text{mg/kg}$  is particularly useful for antagonists that are antibodies or fragments thereof. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. The compounds may be administered concurrently or sequentially or a combination thereof. For example, the TNF- $\alpha$  antagonist may be dosed initially and then followed by administration of the LFA-1 antagonist. Alternatively, the LFA-1 antagonist may be dosed initially and then followed by administration of the TNF- $\alpha$  antagonist. The antagonists may be dosed, for example, daily or every other day for a period of a few (2-4) days or for several (2-6) weeks during a single course of therapy. As noted above, repeated courses of therapy may be administered until the desired suppression of disease or disorder are suppressed.

It is anticipated that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue, may necessitate delivery in a manner different from that to another organ or tissue. ENBREL is supplied as a sterile, white, preservative-free, lyophilized powder for parenteral administration after reconstitution with 1 ml of the supplied Sterile Bacteriostatic Water for Injection, USP (containing 0.9% benzyl alcohol).

In one embodiment, the administration of an LFA-1 antagonist and a TNF- $\alpha$  antagonist provides an improved treatment of an LFA-1 mediated or a TNF- $\alpha$  mediated disorder relative to treatment with either one of the individual compounds alone. That is, treatment with both compounds provides a reduction in the incidence of disease or disorder symptoms relative to a control, for example, which is lower than the reduction in disease or disorder incidence relative to a control, for administration of either compound alone. Such improved efficacy is evidence of a synergistic action of the compounds of the invention in treating an LFA-1 mediated or a TNF- $\alpha$

mediated disorder. The synergism is particularly surprising for LFA-1 antagonists, for example anti-CD11a antibodies, which do not deplete T-cells.

The LFA-1 antagonist and TNF- $\alpha$  antagonist can be administered concurrently with other therapy. For example, a patient being treated for RA can be administered both these antagonists in conjunction with or in addition to conventional drugs used in RA such as methotrexate, glucocorticoids, salicylates, nonsteroidal anti-inflammatory drugs (NSAIDS), or analgesics.

### C. Compositions

The compounds of the invention can be administered for the treatment of LFA-1 and TNF- $\alpha$  mediated disorders in the form of pharmaceutical compositions. Additionally, lipofections or liposomes can also be used to deliver the an LFA-1 antagonist or a TNF antagonist into cells and the target area.

Therapeutic formulations of the active molecules employable with the invention are prepared for storage by mixing the active molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]). Such therapeutic formulations can be in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and *m*-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>®</sup>, PLURONICS<sup>®</sup> or polyethylene glycol (PEG).

In order for the formulations to be used *in vivo* administration, they must be sterile. The formulation may be readily rendered sterile by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The formulations used herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are present in combinations and amounts that are effective for the intended purpose.

The an LFA-1 antagonist or a TNF-A antagonist molecules by also be prepared by entrapping in microcapsules prepared, for example by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively. Such preparations can be administered in colloidal drug delivery systems (for example, liposomes, albumin

microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th Edition (or newer), Osol A. Ed. (1980).

Where sustained-release or extended-release administration of the an LFA-1 antagonist or a TNF-A antagonist is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of such polypeptides, microencapsulation is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon- $\alpha$ , - $\beta$ , - $\gamma$  (rhIFN- $\alpha$ , - $\beta$ , - $\gamma$ ), interleukin-2, and MN rgp120. Johnson *et al.*, *Nat. Med.* 2: 795-799 (1996); Yasuda, *Biomed. Ther.* 27: 1221-1223 (1993); Hora *et al.*, *Bio/Technology* 8: 755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems" in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, eds., (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399 and U.S. Pat. No. 5,654,010.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the active molecule, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include one or more polyanhydrides (*e.g.* U.S.P. 4,891,225; 4,767,628), polyesters such as polyglycolides, polylactides and polylactide-co-glycolides (*e.g.* U.S.P. 3,773,919; U.S.P. 4,767,628; U.S.P. 4,530,840; Kulkarni *et al.*, *Arch. Surg.* 93: 839 (1966)), polyamino acids such as polylysine, polymers and copolymers of polyethylene oxide, polyethylene oxide acrylates, polyacrylates, ethylene-vinyl acetates, polyamides, polyurethanes, polyorthoesters, polyacetylnitriles, polyphosphazenes, and polyester hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), cellulose, acyl substituted cellulose acetates, non-degradable polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinylimidazole), chlorosulphonated polyolefins, polyethylene oxide, copolymers of L-glutamic acid and  $\gamma$ -ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. Additional non-biodegradable polymers which may be employed are polyethylene, polyvinyl pyrrolidone, ethylene vinylacetate, polyethylene glycol, cellulose acetate butyrate and cellulose acetate propionate.

Alternatively, sustained release formulations may be composed of degradable biological materials. Biodegradable polymers are attractive drug formulations because of their biocompatibility, high responsibility for specific degradation, and ease of incorporating the active drug into the biological matrix. For example, hyaluronic acid (HA) may be crosslinked and used as a swellable polymeric delivery vehicle for biological materials. U.S.P. 4,957,744; Valle *et al.*, *Polym. Mater. Sci. Eng.* 62: 731-735 (1991). HA polymer grafted with polyethylene glycol has also been prepared as an improved delivery matrix which reduced both undesired drug leakage and the denaturing associated with long term storage at physiological conditions. Kazuteru, M., *J. Controlled Release* 59: 77-86 (1999). Additional biodegradable polymers which may be used are poly(caprolactone), polyanhydrides, polyamino acids, polyorthoesters, polycyanoacrylates, poly(phosphazines), poly(phosphodiesteres), polyesteramides, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, degradable and nontoxic polyurethanes, polyhydroxylbutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), chitin and chitosan.

Alternatively, biodegradable hydrogels may be used as controlled release delivery vehicles for biological materials and drugs. Through the appropriate choice of macromers, membranes can be produced with a range of permeability, pore sizes and degradation rates suitable for a wide variety of biomolecules.

Alternatively, sustained-release delivery systems for biological materials and drugs can be composed of  
5 dispersions. Dispersions may further be classified as either suspensions or emulsions. In the context of delivery vehicles for biological materials, suspensions are a mixture of very small solid particles which are dispersed (more or less uniformly) in a liquid medium. The solid particles of a suspension can range in size from a few nanometers to hundreds of microns, and include microspheres, microcapsules and nanospheres. Emulsions, on the other hand, are a mixture of two or more immiscible liquids held in suspension by small quantities of  
10 emulsifiers. Emulsifiers form an interfacial film between the immiscible liquids and are also known as surfactants or detergents. Emulsion formulations can be both oil in water (o/w) wherein water is in a continuous phase while the oil or fat is dispersed, as well as water in oil (w/o), wherein the oil is in a continuous phase while the water is dispersed. One example of a suitable sustained-release formulation is disclosed in WO 97/25563. Additionally, emulsions for use with biological materials include multiple emulsions, microemulsions,  
15 microdroplets and liposomes. Microdroplets are unilamellar phospholipid vesicles that consist of a spherical lipid layer with an oil phase inside. *E.g.*, U.S.P. 4,622,219 and U.S.P. 4,725,442. Liposomes are phospholipid vesicles prepared by mixing water-insoluble polar lipids with an aqueous solution.

Alternatively, the sustained-release formulations of an LFA-I antagonist or a TNF-A antagonist may be developed using poly-lactic-coglycolic acid (PLGA), a polymer exhibiting a strong degree of biocompatibility  
20 and a wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, are cleared quickly from the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. For further information see Lewis, "Controlled Release of Bioactive Agents from Lactide/Glycolide polymer," in *Biodegradable Polymers as Drug Delivery Systems* M. Chasin and R. Langeer, editors (Marcel Dekker: New York, 1990), pp. 1-41.

When encapsulated polypeptides remain in the body for a long time, they may denature or aggregate as  
25 a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic  
30 solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

The encapsulated polypeptides or polypeptides in extended-release formulation may be imparted by formulating the polypeptide with a "water-soluble polyvalent metal salts" which are non-toxic at the release concentration and temperature. Exemplary "polyvalent metals" include alkaline earth metals (*e.g.*,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  
35  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Sn}^{4+}$ ,  $\text{Al}^{3+}$  and  $\text{Al}^{3+}$ ). Exemplary anions which form water soluble salts with the above polyvalent metal cations include those formed by inorganic acids and/or organic acids. Such water-soluble salts have a solubility in water (at 20°C) of at least about 20 mg/ml, alternatively 100 mg/ml, alternatively 200 mg/ml.

Suitable inorganic acids that can be used to form the "water soluble polyvalent metal salts" include  
40 hydrochloric, sulfuric, nitric, thiocyanic and phosphoric acid. Suitable organic acids that can be used include aliphatic carboxylic acid and aromatic acids. Aliphatic acids within this definition may be defined as saturated or

unsaturated C<sub>2-9</sub> carboxylic acids (*e.g.*, aliphatic mono-, di- and tri-carboxylic acids). For example, exemplary monocarboxylic acids within this definition include the saturated C<sub>2-9</sub> monocarboxylic acids acetic, propionic, butyric, valeric, caproic, enanthic, caprylic pelargonic and capryonic, and the unsaturated C<sub>2-9</sub> monocarboxylic acids acrylic, propiolic methacrylic, crotonic and isocrotonic acids. Exemplary dicarboxylic acids include the saturated C<sub>2-9</sub> dicarboxylic acids malonic, succinic, glutaric, adipic and pimelic, while unsaturated C<sub>2-9</sub> dicarboxylic acids include maleic, fumaric, citraconic and mesaconic acids. Exemplary tricarboxylic acids include the saturated C<sub>2-9</sub> tricarboxylic acids tricarballic and 1,2,3-butanetricarboxylic acid. Additionally, the carboxylic acids of this definition may also contain one or two hydroxyl groups to form hydroxy carboxylic acids. Exemplary hydroxy carboxylic acids include glycolic, lactic, glyceric, tartronic, malic, tartaric and citric acid. Aromatic acids within this definition include benzoic and salicylic acid.

Commonly employed water soluble polyvalent metal salts which may be used to help stabilize the encapsulated polypeptides of this invention include, for example: (1) the inorganic acid metal salts of halides (*e.g.*, zinc chloride, calcium chloride), sulfates, nitrates, phosphates and thiocyanates; (2) the aliphatic carboxylic acid metal salts calcium acetate, zinc acetate, calcium propionate, zinc glycolate, calcium lactate, zinc lactate and zinc tartrate; and (3) the aromatic carboxylic acid metal salts of benzoates (*e.g.*, zinc benzoate) and salicylates.

#### D. Therapeutic Utility

It is contemplated that the compounds of the invention may be used to treat various LFA-1 and/or TNF- $\alpha$  mediated diseases or disorders, including degenerative cartilagenous disorders such as rheumatoid arthritis, juvenile chronic arthritis (*e.g.*, Polyarticular-Course Juvenile Rheumatoid Arthritis (JRA)) and spondyloarthropathies. RA refractory to or intolerant of methotrexate can also be treated with the LFA-1 and TNF- $\alpha$  antagonists of the invention.

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes: the joint space/fluid is infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs in two major forms. One form is the development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin ulcers and gangrene. Patients often also develop rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations which can occur in RA include: pericarditis, pleuritis, coronary arteritis, interstitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rheumatoid nodules.

Juvenile chronic arthritis is a chronic idiopathic inflammatory disease which begins often at less than 16 years of age. Its phenotype has some similarities to RA: some patients which are rheumatoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic anterior uveitis and systemic amyloidosis.

The degenerative cartilagenous disorder osteoarthritis (OA) is a localized degenerative disease that affects the articular structure and results in pain and diminished function. OA is characterized by perturbations in the cartilage surface followed by clefts and fibrillations and finally by loss of the entire thickness of the cartilage layer. Additional symptoms of OA include the formation of calcified outgrowths of the periarticular bone and disfigurement coincident with assymetric cartilage destruction. OA may be be classified into two types: primary and secondary. Primary OA refers to the spectrum of degenerative joint diseases for which no underlying etiology has been determined. Typically, the joint affected by primary OA are the interphalangeal joints of the hands, the first carpometacarpal joints, the hips, the knees, the spine, and some joints in the midfoot. Interestingly, large joints, such as the ankles, elbows and shoulders tend to be spared in primary OA. In contrast, secondary OA occurs as a result of defined injury. Secondary OA is often associated with metabolic diseases such as hemochromatosis and alkaptonuria, developmental abnormalities such as developmental dysplasia of the hips (congenital dislocation of the hips) and limb-length discrepancies, including trauma, inflammatory arthritides such as rheumatoid arthritis or gout, septic arthritis, and neuropathic arthritis.

Injuries to cartilage fall into three categories: (1) microdamage or blunt trauma, (2) chondral fractures, and (3) osteochondral fractures.

Microdamage to chondrocytes and cartilage matrix may be caused by a single impact or through repetitive blunt trauma. Chondral fractures are characterized as a disruption of the articular surface without violation of the subchondral plate. Chondrocyte necrosis at the injury site occurs, followed by increased mitotic and metabolic activity of the surviving chondrocytes bordering the injury within a few days of injury. This is followed by fibrous tissue forming a lining of clefts in the surface. There is increased synthesis of extracellular matrix components and type II collagen for about two weeks after injury, after which the anabolism returns to normal. However, the transitory increase in mitotic and metabolic activity and the repair response resulting therefrom is suboptimal - resulting in the formation of fibrocartilage. Osteochondral fractures, the most serious of the three type of injuries, are lesions crossing the tidemark, or the underlying subchondral plate. In this type of injury, the presence of subchondral vasculature elicits the three-phase response typically encountered in vascular tissues: (1) necrosis; (2) inflammation; and (3) repair. Initially the lesion fills with blood and clots. The resulting fibrin clot activates an inflammatory response and becomes vascularized repair tissue, and the various cellular components release growth factors and cytokines including transforming growth factor beta (TGF-beta), platelet-derived growth factor (PDGF), bone morphogenic proteins, and insulin-like growth factors. Buckwalter *et al.*, *J. Am. Acad. Orthop. Surg.* 2: 191-201 (1994).

Spondyloarthropathies are a group of disorders with some common clinical features and the common association with the expression of HLA-B27 gene product. The disorders include: ankylosing spondylitis, Reiter's syndrome (reactive arthritis), arthritis associated with inflammatory bowel disease, spondylitis associated with psoriasis, juvenile onset spondyloarthropathy and undifferentiated spondyloarthropathy. Distinguishing features include sacroileitis with or without spondylitis; inflammatory asymmetric arthritis; association with



HLA-B27 (a serologically defined allele of the HLA-B locus of class I MHC): ocular inflammation, and absence of autoantibodies associated with other rheumatoid disease. The cell most implicated as key to induction of the disease is the CD8+ T lymphocyte, a cell which targets antigen presented by class I MHC molecules. CD8+ T cells may react against the class I MHC allele HLA-B27 as if it were a foreign peptide expressed by MHC class I molecules. It has been hypothesized that an epitope of HLA-B27 may mimic a bacterial or other microbial antigenic epitope and thus induce a CD8+ T cells response.

Other LFA-1 and/or TNF- $\alpha$  mediated diseases or disorders which may be treated with the combination of the invention include (1) TNF- $\alpha$  mediated diseases or disorders such as (A) acute and chronic immune and autoimmune pathologies, such as systemic lupus erythematosus (SLE) rheumatoid arthritis, thyroidosis, graft versus host disease, scleroderma, diabetes mellitus, Graves' disease, and the like; (B) infections, including, but not limited to, sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, acute and chronic parasitic and/or infectious diseases, bacterial, viral or fungal, such as a HIV, AIDS (including symptoms of cachexia, autoimmune disorders, AIDS dementia complex and infections); (C) inflammatory diseases, such as chronic inflammatory pathologies and vascular inflammatory pathologies, including chronic inflammatory pathologies such as sarcoidosis, chronic inflammatory bowel disease, ulcerative colitis, and Crohn's pathology and vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, and Kawasaki's pathology; (D) neurodegenerative diseases, including, but are not limited to, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders' such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranucleo palsy; Cerebellar and Spinocerebellar Disorders, such as astructural lesions of the cerebellum; spinocerebellar degenerations (spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and MachadoJoseph)); and systemic disorders (Refsum's disease, abetalipoproteinemia, ataxia, telangiectasia, and mitochondrial multi.system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerorden-Spatz disease; and Dementia pugilistica, or any subset thereof; and (2) LFA-1 mediated diseases or disorders such as T cell inflammatory responses such as inflammatory skin diseases including psoriasis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); adult respiratory distress syndrome; dermatitis; meningitis; encephalitis; uveitic; allergic conditions such as eczema and asthma and other conditions involving infiltration of T cells and chronic inflammatory responses; skin hypersensitivity reactions (including poison ivy and poison oak); atherosclerosis; leukocyte adhesion deficiency; autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), diabetes mellitus, multiple sclerosis, Reynaud's syndrome, autoimmune thyroiditis, experimental autoimmune encephalomyelitis, Sjorgen's syndrome, juvenile onset diabetes, and immune responses associated with delayed hypersensitivity mediated by cytokines and T-lymphocytes typically found in tuberculosis.

sarcoidosis, polymyositis, granulomatosis and vasculitis; pernicious anemia; diseases involving leukocyte diapedesis; CNS inflammatory disorder, multiple organ injury syndrome secondary to septicemia or trauma; autoimmune haemolytic anemia; myethemia gravis; antigen-antibody complex mediated diseases; all types of transplantations, including graft vs. host or host vs. graft disease; etc.

5            Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is induced by an active inflammatory process. Scleroderma can be localized or systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An immunologic basis is implied by the presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear  
10            antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin lesions suggesting that T cell interaction with these cells may have a role in the pathogenesis of the disease. Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and  
15            hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis, scarring/fibrosis.

             Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-specific  
20            autoantibodies are directed against and inhibit the function of components, proteins and RNAs, involved in protein synthesis.

             Sjögren's syndrome is the result of immune-mediated inflammation and subsequent functional destruction of the tear glands and salivary glands. The disease can be associated with or accompanied by inflammatory connective tissue diseases. The disease is associated with autoantibody production against Ro and  
25            La antigens, both of which are small RNA-protein complexes. Lesions result in keratoconjunctivitis sicca, xerostomia, with other manifestations or associations including biliary cirrhosis, peripheral or sensory neuropathy, and palpable purpura.

             Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by the affected vessels and  
30            eventual end-organ dysfunction in some cases. Vasculitides can also occur as a secondary lesion or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, etc, particularly in diseases also associated with the formation of immune complexes. Diseases in the primary systemic vasculitis group include: systemic necrotizing vasculitis: polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis. Miscellaneous  
35            vasculitides include: mucocutaneous lymph node syndrome (MLNS or Kawasaki's disease), isolated CNS vasculitis, Behet's disease, thromboangiitis obliterans (Buerger's disease) and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or both.

40            Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis

involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

5 Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal nocturnal hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

10 In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

Type I diabetes mellitus or insulin-dependent diabetes is the autoimmune destruction of pancreatic islet  $\beta$  cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

20 Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition of antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune mechanisms result in inflammatory response that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

30 Demyelinating diseases of the central and peripheral nervous systems, including multiple sclerosis: idiopathic demyelinating polyneuropathy or Guillain-Barre syndrome; and Chronic Inflammatory Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is evidence to suggest that disease induction and progression is dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition, environment, and autoimmunity all contribute. Lesions contain infiltrates of predominantly T lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely T lymphocyte driven.

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias: Idiopathic Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a dysregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

Psoriasis is a T lymphocyte-mediated inflammatory disease characterized by hyperproliferation of keratinocytes and accumulation of activated T cells in the epidermis and dermis of psoriatic lesions. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Transplantation associated diseases, including graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E and herpes) bacterial infection, fungal infections, and protozoal and parasitic infections (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response to infectious agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (*i.e.* as from chemotherapy) immunodeficiency, and neoplasia.

Additionally, inhibition of molecules with proinflammatory properties may have therapeutic benefit in reperfusion injury; stroke; myocardial infarction; atherosclerosis; acute lung injury; hemorrhagic shock; burn; sepsis/septic shock; acute tubular necrosis; endometriosis; degenerative joint disease and pancreatitis.

#### E. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and an instruction. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is, for example, effective for treating an LFA-1 and/or TNF- $\alpha$  mediated disorder, for example a degenerative cartilaginous disorder, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition will be an LFA-1 antagonist and/or a TNF-A antagonist. The composition can comprise any or multiple ingredients disclosed herein. The instruction on, or associated with, the container indicates that the composition is used for treating the condition of choice. For example, the instruction could indicate that the composition is effective for the treatment of osteoarthritis arthritis, rheumatoid arthritis any other degenerative cartilaginous disorder, or any other LFA-1 and/or TNF- $\alpha$  mediated disorder. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. Alternatively, the composition may contain any of the carriers, excipients and/or stabilizers mentioned herein under section *E. Pharmaceutical Compositions and Dosages*. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

#### F. Assays/models

Assays and animal models are useful to evaluate the activity of the combination of compounds used in the methods of the invention. Some assays and models useful in assessing the effectiveness of the compounds in the treatment of joint disease, the repair of cartilage and the treatment of degenerative cartilagenous disorders are described below.

##### Collagen induced arthritis assay/model

Rheumatoid arthritis (RA) is an immune disorder which appears to involve production of auto-antibodies. Antibodies to a protein expressed exclusively in cartilage, namely type II collagen, are present in the synovial fluid of some RA patients. Trentham, D.E *et al.*, *Arthrit. Rheum.* 24: 1363-9 (1981). However, these antibodies are not necessarily the cause of the disease, but rather may be secondary to the inflammation. Injection of type II collagen into animals creates a specific immune reaction within synovial joints.

The collagen-induced arthritis (CIA) model is considered a suitable model for studying potential drugs or biologics active in human arthritis because of the many immunological and pathological similarities to human RA, the involvement of localized major histocompatibility, complete class-II-restricted T helper lymphocyte activation, and the similarity of histological lesions. Features of this CIA model which are similar to that found in RA patients include: erosion of cartilage and bone at joint margins (as can be seen in radiographs), proliferative synovitis, symmetrical involvement of small and medium-sized peripheral joints in the appendicular, but not the axial, skeleton. Jamieson, T.W. *et al.*, *Invest. Radiol.* 20: 324-9 (1985). Furthermore, IL-1 and TN- $\alpha$  appear to be involved in CIA as in RA. Joosten *et al.*, *J. Immunol.* 163: 5049-5055, (1999). TNF neutralizing antibodies and separately, TNFR:Fc reduced the symptoms of RA in this model (see Williams *et al.*, PNAS Oct. 1992, 89:9784-9788; Wooley *et al.*, 1993, *J. Immunol.* 151: 6602-6607). Further evidence of the CIA model being predictive of the human condition and response to treatment in RA can be seen, e.g., from the clinical results with ENBREL. The model is described in greater detail in the examples.

In this model for rheumatoid arthritis, type II collagen is purified from bovine articular cartilage (Miller, 1972, *Biochemistry* 11:4903) and used to immunized mice (Williams *et al.*, 1994, *Natl. Acad. Sci. USA* 91:2762). Symptoms of arthritis include erythema and/or swelling of the limbs as well as erosions or defects in cartilage and bone as determined by histology. This widely used model is also described, for example, by Holmdahl *et al.* 1989, *APMIS* 97:575.

##### Articular cartilage explant assay

In this assay, the synthetic and prophylactic potential of the test compounds on the cartilage matrix is described. To this end, proteoglycan (PG) synthesis and breakdown are measured, as well as the release of nitric oxide. Proteoglycans are the second largest component of the organic material in articular cartilage (Kuettnner, K.E. *et al.*, *Articular Cartilage Biochemistry*, Raven Press, New York, USA (1986), p.456; Muir, H., *Biochem. Soc. Tran.* 11: 613-622 (1983); Hardingham, T.E., *Biochem. Soc. Trans.* 9:489-497 (1981). Since proteoglycans help determine the physical and chemical properties of cartilage, the decrease in cartilage PGs which occurs during joint degeneration leads to loss of compressive stiffness and elasticity, an increase in hydraulic permeability, increased water content (swelling), and changes in the organization of other extracellular components such as collagens. Thus, PG loss is an early step in the progression of degenerative cartilagenous disorders, one which further perturbs the biomechanical and biochemical stability of the joint. PGs in articular

cartilage have been extensively studied because of their likely role in skeletal growth and disease. Mow, V.C., & Ratcliffe, A. *Biomaterials* **13**: 67-97 (1992). Proteoglycan breakdown, which is increased in diseased joints, is measured in the assays described herein by quantitating PGs in the media of explants using the colorimetric DMMB assay. Farndale and Buttle, *Biochem. Biophys. Acta* **883**: 173-177 (1985). Incorporation of  $^{35}\text{S}$ -sulfate into proteoglycans is used as an indication of proteoglycan synthesis.

The evidence linking IL-1 $\alpha$  and degenerative cartilagenous diseases is substantial. For example, high levels of interleukin-1 $\alpha$  (IL-1 $\alpha$ )(Pelletier JP *et al.*, "Cytokines and inflammation in cartilage degradation" in *Osteoarthritic Edition of Rheumatic Disease Clinics of North America*, Eds. RW Moskowitz, Philadelphia, W.D. Saunders Company, 1993, p.545-568) and IL-1 receptors (Martel-Pelletier *et al.*, *Arthritis Rheum.* **35**: 530-540 (1992) have been found in diseased joints, and IL-1 $\alpha$  induces cartilage matrix breakdown and inhibits synthesis of new matrix molecules. Baragi *et al.*, *J. Clin. Invest.* **96**: 2454-60 (1995); Baragi *et al.*, *Osteoarthritis Cartilage* **5**: 275-82 (1997); Evans *et al.*, *J. Leukoc. Biol.* **64**: 55-61 (1998); Evans *et al.*, *J. Rheumatol.* **24**: 2061-63 (1997); Kang *et al.*, *Biochem. Soc. Trans.* **25**: 533-37 (1997); Kang *et al.*, *Osteoarthritis Cartilage* **5**: 139-43 (1997). Because of the association of IL-1 $\alpha$  and IL-1 receptors with diseased tissue, also assayed are the effects of the test compound in the presence of IL-1 $\alpha$ . The ability of the test compound to not only have positive effects on cartilage, but also to counteract the catabolic effects of IL-1 $\alpha$ , is strong evidence of the protective effect exhibited by the test compound. In addition, such an activity suggests that the test compound could inhibit the degradation which occurs in arthritic conditions, since antagonism of IL-1 $\alpha$  function has been shown to reduce the progression of osteoarthritis. Arend, W.P. *et al.*, *Ann. Rev. Immunol.* **16**: 27-55 (1998).

The production of nitric oxide (NO) can be induced in cartilage by catabolic cytokines such as IL-1. Palmer, RMJ *et al.*, *Biochem. Biophys. Res. Commun.* **193**: 398-405 (1993). NO has also been implicated in the joint destruction which occurs in arthritic conditions. Ashok *et al.*, *Curr. Opin. Rheum.* **10**: 263-268 (1998). Unlike normal (undiseased or uninjured) cartilage, cartilage obtained from osteoarthritic joints produces significant amounts of nitric oxide *ex vivo*, even in the absence of added stimuli such as interleukin-1 or lipopolysaccharide. *In vitro*, nitric oxide exerts detrimental effects on chondrocyte function, including inhibition of collagen and proteoglycan synthesis, enhanced apoptosis and inhibition of adhesion to the extracellular matrix. Nitrite concentrations have been shown to be higher in synovial fluid from osteoarthritic patients than in fluid from rheumatoid arthritic patients. Renoux *et al.*, *Osteoarthritis Cartilage* **4**: 175-179 (1996). Furthermore, animal models suggest that inhibition of nitric oxide production reduces progression of arthritis. Pelletier, JP *et al.*, *Arthritis Rheum* **7**:1275-86 (1998); van de Loo *et al.*, *Arthritis Rheum.* **41**:634-46 (1998); Stichtenoth, DO & Frolich J.C. *Br. J. Rheumatol.* **37**: 246-57 (1998). Since NO also has effects on other cells, the presence of NO within the articular joint could increase vasodilation and permeability, potentiate cytokines release by leukocytes, and stimulate angiogenic activity by monocyte-macrophages. Thus, production of NO by cartilage correlates with a diseased state, and since NO appears to play a role in both the erosive and the inflammatory components of joint diseases, a factor which decreases nitric oxide production would likely be beneficial for the treatment of degenerative cartilaginous disorders.

The assay described herein is based on the principle that 2,3-diaminonaphthalene (DAN) reacts with nitrite under acidic conditions to form 1-(H)-naphthotriazole, a fluorescent product which can be quantified. As NO is quickly metabolized into nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), detection of nitrite is one means of detecting (albeit undercounting) the actual NO produced in cartilagenous tissue.

Mouse Patellae assay

This experiment examines the effects of the test compound on proteoglycan synthesis in the patellae (knee caps) of mice. This assay uses intact cartilage (including the underlying bone) and thus tests factors under conditions which approximate the *in vivo* environment of cartilage. Compounds are either added to patellae *in vitro*, or are injected into knee joints *in vivo* prior to analysis of proteoglycan synthesis in patellae *ex vivo*. As has been shown previously, *in vivo* treated patellae show distinct changes in PG synthesis *ex vivo*. (Van den Berg *et al.*, *Rheum. Int.* 1: 165-9 (1982); Vershure, P.J. *et al.*, *Ann. Rheum. Dis.* 53: 455-460 (1994); and Van de Loo *et al.*, *Arthrit. Rheum.* 38: 164-172 (1995). In this model, the contralateral joint of each animal can be used as a control. The procedure is described in greater detail in the examples.

Guinea pig model

This assay measures the effects of the test compound on both the stimulation of *ex vivo* PG synthesis and inhibition of *ex vivo* PG release in an model from the cartilage matrix of the Dunkin Hartley (DH) Guinea Pig, an accepted animal model for osteoarthritis. Young *et al.*, "Osteoarthritis", *Spontaneous animal models of human disease* vol. 2, pp. 257-261, Acad. Press, New York, (1979); Bendele *et al.*, *Arthritis Rheum.* 34: 1180-1184; Bendele *et al.*, *Arthritis Rheum.* 31: 561-565 (1988); Jimenez *et al.*, *Laboratory Animal Sciences* vol. 47 (6): 598-601 (1997).

The DH guinea pigs develop arthritic lesions resembling those of human osteoarthritis (OA) of the knee and other joints. At 2 months of age, these animals develop mild OA that is detectable by the presence of minimal histologic changes. For example, proteoglycan synthesis is increased, as evidenced by higher levels of PG in the cartilage tissue itself, as well as in the synovial fluid. The disease progresses, and by 16-18 months of age, moderate to severe cartilage degeneration on the medial tibial plateau is observed and at 22 months, the animals are severely impaired with marginal osteophytes of the tibia and femur, sclerosis of the subchondral bone of the tibial plateau, femoral condyle cysts and calcification of the collateral ligaments. Jimenez *et al.*, *supra*.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

EXAMPLES

Examples 1-3 describe the treatment of arthritis with an LFA-1 antagonist (anti-murine CD11a antibody, M17) or TNF antagonist (ENBREL; Etanercept) alone or in combination, in the collagen-induced arthritis (CIA) model. The CIA model is discussed above under Assays/models. Examples 1 and 3 describe treatment of arthritis in two strains of mice, with a combination of anti-murine CD11a antibody (M17) and ENBREL. Example 2 describes treatment with either M17 or ENBREL alone. The preclinical studies for ENBREL used the same animal model.

EXAMPLE 1- Treatment with an LFA-1 antagonist and a TNF antagonist

DBA-1J mice were immunized with 100 ug bovine collagen type II in 100 ul complete Freund's adjuvant (CFA) followed by a second injection of the same collagen in incomplete Freund's adjuvant (IFA) 21 days later. The collagen type II in CFA or IFA was injected intradermally at the base of the tail.

Animals were evaluated every other day. At the onset of arthritis in any of the animals, as noted by swelling of any of the paws, treatment was initiated in all animals placed randomly into the following treatment groups with 12 mice per group.

Group 1. Control: Treatment with saline, 100 ul, intraperitoneal route, every day for 14 days, then 3 times per week every other day (Monday, Wednesday and Friday).

Group 2. Anti-murine CD11a monoclonal antibody M17, 150 ug (approx 8 mg/kg) via intraperitoneal route given at onset of disease followed by 3 times per week every other day (Monday, Wednesday and Friday) until the end of the study.

Group 3. ENBREL (human TNF-Fc, 50 ug) intraperitoneal route, given daily at onset of disease for 14 days.

Group 4. Combination of ENBREL and Anti-CD11a: Anti-murine CD11a mab M17, 150 ug (approximately 8 mg/kg) via intraperitoneal route given at onset of disease followed by 3 times per week every other day (Monday, Wednesday and Friday) until the end of the experiment. ENBREL, intraperitoneal route, given daily at onset of disease for 14 days.

After the onset of treatment, the mice were evaluated every other day 3 times a week and the severity of paw swelling was subjectively graded for each paw on a scale of 0-4 with 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. A cumulative score was recorded for each animal (potential range 0-16). The animals were terminated 38 days after the initiation of treatment. Radiographs were taken on all four limbs to evaluate for joint lesions and the paws were collected for histopathology. The severity of disease as determined by clinical score for each group (mean +/- standard deviation) was graphed and compared between groups. The clinical scores taken on the last day were corroborated by histological and radiologic analysis of all four paws done at the terminus of the study.

The use of either ENBREL or anti-CD11a antibody reduced the clinical scores compared to the control group ( $p < 0.05$ ) and the combination of anti-CD11a antibody and ENBREL improved the clinical scores compared to ENBREL alone ( $p < 0.05$ ). See Figure 1.

EXAMPLE 2- Treatment of arthritis with an LFA-1 antagonist or a TNF antagonist

In this example, arthritis was induced in DBA-1LacJ mice (Figure 2) or DBA-1J mice (Figure 3) which were then treated with anti-murine CD11a antibody (M17), ENBREL, or saline as a control. These experiments were performed as described in Example 1 and in the inset in Figure 2 and Figure 3. Treatment was initiated on day 48 or day 22 post immunization, the day of onset of arthritis in the experiments of Figure 2 and Figure 3 respectively.



The results presented in Figure 2 and Figure 3 show that anti-CD11a antibody or ENBREL alone is effective in treating arthritis as evidenced by the reduction in clinical scores.

#### EXAMPLE 3- Treatment of arthritis with an LFA-1 antagonist and a TNF antagonist

5 In this example, arthritis was induced in DBA-1LacJ mice (Figure 4) or DBA-1J mice (Figure 5) which were then treated with anti-murine CD11a antibody (M17) alone, ENBREL alone, saline as a control, or a combination of M17 and ENBREL. These experiments were performed as described in Example 1 and in the inset in Figures 4 and 5. In Figure 4, treatment was initiated on day 40 post immunization. M17 was given at 160 ug, three times per week for the duration of the study. For the combination therapy, the mice received 50 ug  
10 Enbrel daily up to a total of 14 doses in one experiment, and for the duration of the study in another experiment. In Figure 5, treatment was initiated on day 24 post immunization, the day of onset of arthritis. Enbrel was administered everyday (qd) for 14 days, then every other day (qod), (Monday, Wednesday, Friday) until the end of the experiment.

As is evident from the results shown in Figure 4 and Figure 5, combination therapy with an LFA-1  
15 antagonist and a TNF antagonist had a synergistic effect over treatment with either antagonist alone, resulting in greater reduction in mean clinical scores to almost normal in this animal model.

In Examples 4-6 below, a test compound refers to an LFA-1 antagonist (e.g., anti-CD11a antibody) or a  
20 TNF antagonist (e.g., ENBREL). The volumes, concentrations and time points are exemplary and can be varied as will be familiar to one of skill in the art.

#### EXAMPLE 4 - Articular cartilage explant assay

This assay, discussed above under Assays/Models, examines both the synthetic and prophylactic  
25 potential of a test compound on the cartilage matrix. This potential is determined both by stimulation of matrix synthesis and inhibition of matrix breakdown, as determined by: (1) PG synthesis in the articular matrix; (2) Inhibition of PG release; (3) Inhibition of IL-1 $\alpha$  induced breakdown; and (4) Inhibition of nitric oxide.

##### *Articular cartilage explants*

The metacarpophalangeal joint of 4-6 month old female pigs is aseptically opened, and articular  
30 cartilage is dissected free of the underlying bone. The cartilage is minced, washed and cultured in bulk for at least 24 hours in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> in serum free low glucose 50:50 DMEM:F12 media with 0.1% BSA, 100U/ml penicillin/streptomycin (Gibco), 2mM L-glutamine, 1x GHT, 0.1mM MEM Sodium Pyruvate (Gibco), 20  $\mu$ g/ml Gentamicin (Gibco), 1.25mg/L Amphotericin B (Sigma), 5 $\mu$ g/mL Vitamin E and 10 $\mu$ g/mL transferrin. Approximately 50mg of articular cartilage is aliquoted into Micronics tubes and  
35 incubated for at least 24 hours in above media before being changed into media without Vitamin E and transferrin. Test proteins are then added. Media is harvested and changed at various time points (e.g., 0, 24, 48, 72h).

##### *Measurement of proteoglycans:*

40 DMMB is a dye that undergoes metachromasia (a change in color, in this case from blue to purple) upon

binding to sulfated glycosaminoglycans (GAG), the side-chains of proteoglycans. The addition of sulfated proteoglycans to DMMB causes a decrease in the peak values at 590 and 660 nm with an increase in absorbance at 530 nm. The amount of proteoglycans in media is determined by adding DMMB dye in a 96 well plate format, and the change in color is quantitated using a spectrophotometer (Spectramax 250). The DMMB assay is a well-accepted method to measure the amount of proteoglycans in cartilage cultures. For this assay, a standard curve is prepared using chondroitin sulfate ranging from 0.0 to 5.0  $\mu\text{g}$ . The procedure has been adapted from the colorimetric assay described in Farndale and Buttle, *Biochem. Biophys. Acta* **883**: 173-177 (1986).

#### *Measurement of proteoglycan synthesis in articular cartilage explants*

After the media change at 48 hr,  $^{35}\text{S}$ -sulfate (to a final concentration of 10  $\mu\text{Ci/ml}$ ) is added to the cartilage explants. After an overnight incubation at 37°C, media is saved for measurements of nitric oxide or proteoglycan content. Cartilage pieces are washed two times using explant media. 900  $\mu\text{L}$  digestion buffer containing 10 mM EDTA, 0.1 M Sodium phosphate and 1 mg/ml proteinase K (Gibco BRL) is added to each tube and incubated overnight in a 50°C water bath. 600  $\mu\text{L}$  of the digest is mixed with 600  $\mu\text{L}$  of 10% w/v cetylpyridinium chloride (Sigma). Samples are spun at 1000 x g for 15 min. The supernatant is removed, and 500  $\mu\text{L}$  formic acid (Sigma) is added to the samples to dissolve the precipitate. Solubilized pellets are transferred to scintillation vials containing 10 ml scintillation fluid (ICN), and samples are read in a scintillation counter.

#### *Measurement of nitric oxide (NO)*

10  $\mu\text{L}$  of 0.05 mg/ml 2,3-diaminonaphthalene (DAN) in 0.62M HCl is added to 100  $\mu\text{L}$  media from cartilage explants. Samples are mixed and incubated at room temperature for 10-20 minutes. The reaction is terminated with 5  $\mu\text{L}$  of 2.8 M NaOH. The fluorescent product, 2,3-diaminonaphthotriazole, is measured using a Cytoflor fluorescent plate reader with excitation at 360 nm and emission read at 450 nm.

#### EXAMPLE 5 -Mouse Patellae Assay

This assay determines the *in vivo* effect of an LFA-1 antagonist and a TNF antagonist (e.g., anti-CD11a antibody and ENBRELE) on proteoglycan synthesis in the patellae of mice. The patella is a very useful model because it permits the evaluation of the effects of a test compound on cartilage which has not been removed from the underlying bone. Moreover, the evaluation of localized ambular *in vivo* injections offers virtually ideal experimental controls, since each animal has two patellae in separate and distinct regions of their body. The procedure herein is adapted from the one outlined in Van den Berg *et al.*, *Rheum. Int.* **1**: 165-9 (1982); Vershure P.J. *et al.*, *Ann. Rheum. Dis.* **53**: 455-460 (1994); and Van de Loo *et al.*, *Arthrit. Rheum.* **38**: 164-172 (1995). This assay is discussed above under Assays/Models.

In the *ex vivo* treatment group, the patellae of mice are carefully removed and incubated overnight in media with one of the following: no additional factors (e.g., saline control); IL-1 (e.g., at 100 ng/ml); anti-CD11a antibody or ENBRELE; IL-1 and anti-CD11a antibody or IL-1 and ENBRELE; anti-CD11a antibody and ENBRELE in combination; to look for the ability of the test compound(s) to inhibit the effects of IL-1. During the last 3 hours of the incubation, 30  $\mu\text{Ci/ml}$   $^{35}\text{S}$ -sulfur is added for 3 hours in a tissue culture incubator followed by three washings with PBS. Samples are then fixed overnight in 10% formalin followed by decalcifying in 5% formic acid for at least 5 hours. The cartilage is dissected away from the underlying bone and placed in

500  $\mu$ l of solvent and incubated at 60°C for 1.5 hours. 10 ml of HIONIC-fluor is added to each tube and mixed thoroughly. The solution is transferred into scintillation vials and  $^{35}\text{S}$  uptake as a measure of PG synthesis is then determined on a scintillation counter.

In the *in vivo* treatment group, animals are separated into two subgroups and injected (e.g., into knee joints) with the test compounds individually or in combination (e.g., anti-CD11a antibody and ENBREL) into one knee. The dosage and dosing regimen is varied to define the optimum conditions for treatment. The patellae are then harvested and assayed as described above.

#### EXAMPLE 6 -Guinea pig model

This guinea pig model is an accepted animal model for osteoarthritis and is useful for measuring the effects of a test compound on both the stimulation of proteoglycan (PG) synthesis and inhibition of PG release from the cartilage matrix of the Dunkin Hartley (DH) Guinea Pig.

Male Dunkin Hartley guinea pigs are obtained from Charles River Laboratories (Wilmington, MA) and group-housed. The animals are separated into treatment groups for sacrifice at 1-2, 6 and 11 months of age. The animals are treated with an the aforementioned antagonists alone or in combination, e.g., as described in Example 1. Appropriate controls (e.g., saline injection alone) are included. At sacrifice, the metacarpophalangeal joints are aseptically dissected, and the articular cartilage is removed by free-hand slicing taking care so as to avoid the underlying bone. The cartilage is minced, washed and cultured in bulk for at least 24 hours in a humidified atmosphere of 95% air and 5%  $\text{CO}_2$  in serum free (SF) LG DMEM/F12 media with 0.1% BSA, 100 U/ml penicillin/streptomycin (Gibco), 2 mM L-Glutamine, 1x GHT, 0.1 mM MEM sodium pyruvate (Gibco), 20  $\mu\text{g}/\text{ml}$  Genamicin (Gibco) and 1.25 mg/L Amphotercin B. Articular cartilage is aliquoted into Micronics tubes (approximately 55 mg per tube) and incubated for at least 24 hours in the above media. The media is harvested and changed at various time points (0, 24, 48 and 72 hours).

#### Proteoglycan release:

Media harvested at various time points is assayed for proteoglycan content using the 1,9-dimethylmethylene blue (DMB) colorimetric assay of Farndale and Buttle. *Biochem. Biophys. Acta* **883**: 173-177 (1985). A standard curve is prepared of chondroitin sulfate ranging from 0.0 to 5.0 mg.

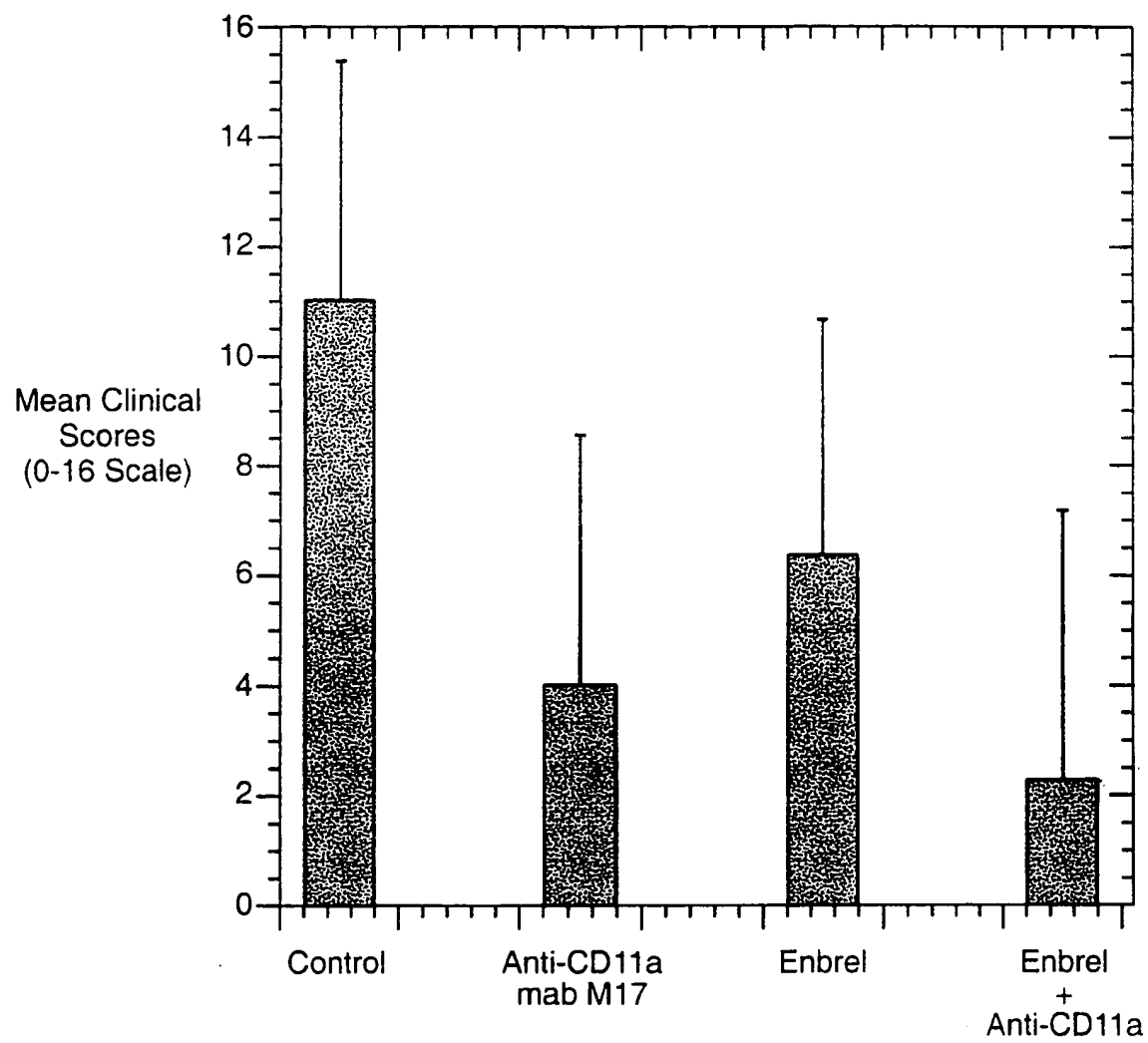
#### Measurement of proteoglycan synthesis:

After the media change at 48 hours, a final concentration of 10 mCi/ml  $^{35}\text{S}$  is added to the cartilage explant culture. After an additional 17 hours of incubation at 37°C, media is saved for subsequent PG and NO analysis. Cartilage pieces are washed two times using explant media. 900  $\mu\text{l}$  digestion buffer containing 10 mM EDTA, 0.1 M Sodium phosphate and 1 mg/ml proteinase K (Gibco BRL) is added to each tube and incubated overnight in a 50°C water bath. 600  $\mu\text{L}$  of the digest is mixed with 600  $\mu\text{L}$  of 10% w/v cetylpyridinium chloride (Sigma). Samples are spun at 1000 x g for 15 min. The supernatant is removed, and 500  $\mu\text{L}$  formic acid (Sigma) is added to the samples to dissolve the precipitate. Solubilized pellets are transferred to scintillation vials containing 10 ml scintillation fluid (ICN), and samples are read in a scintillation counter.

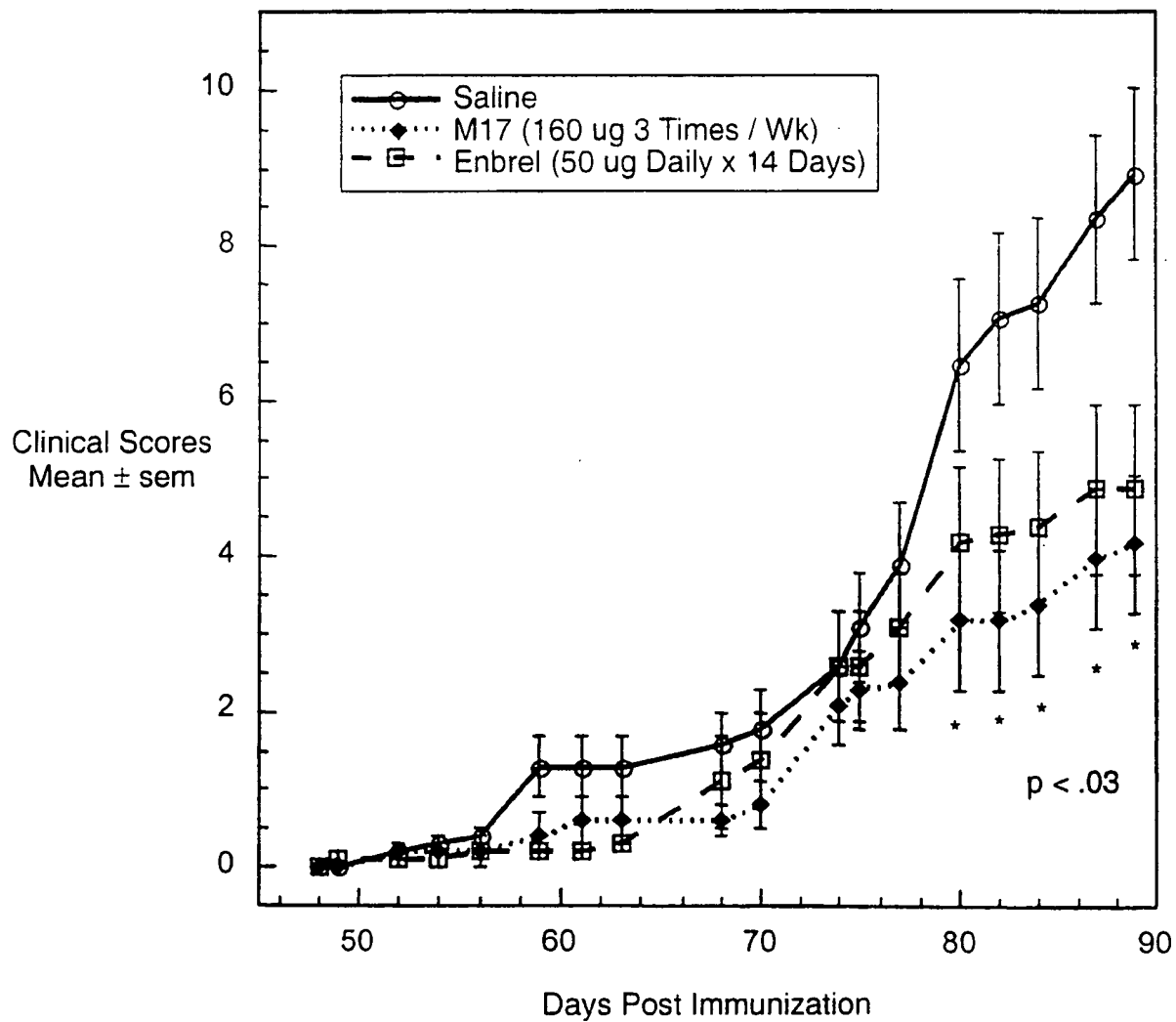
## Claims:

1. A method of treating an LFA-1 or a TNF- $\alpha$  mediated disorder, comprising administering to a mammal in need thereof effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist.
- 5 2. A method of treating cartilage damage from injury or preventing initial or continued damage by a degenerative cartilagenous disorder or injury, comprising contacting the cartilage with effective amounts of an LFA-1 antagonist and a TNF- $\alpha$  antagonist.
3. The method of claim 1 or 2, wherein the disorder is a degenerative cartilagenous disorder.
4. The method of claim 3, wherein the degenerative cartilagenous disorder is selected from the group  
10 consisting of rheumatoid arthritis and osteoarthritis.
5. The method of one of claims 1-4, wherein the LFA-1 antagonist is an anti-LFA-1 antibody, preferably an anti-CD11a antibody.
6. The method of one of claims 1-5, wherein the LFA-1 antagonist is a non T-cell depleting anti-CD11a antibody.
- 15 7. The method of one of claims 1-6, wherein the TNF- $\alpha$  antagonist is an immunoadhesin.
8. The method of one of claim 7 wherein the immunoadhesin is a fusion of at least a portion of a TNF- $\alpha$  binding protein and a portion of an immunoglobulin.
9. The method of one of claim 8, wherein the TNF- $\alpha$  binding protein is a TNF- $\alpha$  receptor - IgG Fc fusion protein.
- 20 10. A composition, comprising an LFA-1 antagonist and a TNF- $\alpha$  antagonist.
11. The composition of claim 10, wherein the LFA-1 antagonist is an anti-LFA-1 antibody
12. The composition of claim 11, wherein the anti-LFA-1 antibody is an anti-CD11a antibody.
13. The composition of claim 12, wherein the an anti-CD11a antibody is a non T-cell depleting antibody.
14. The composition of claim 10, wherein the TNF- $\alpha$  antagonist is an immunoadhesin.
- 25 15. The composition of claim 14, wherein the immunoadhesin is a fusion of at least a portion of a TNF- $\alpha$  binding protein and a portion of an immunoglobulin.
16. The composition of claim 15, wherein the TNF- $\alpha$  binding protein is a TNF- $\alpha$  receptor - IgG Fc fusion protein.

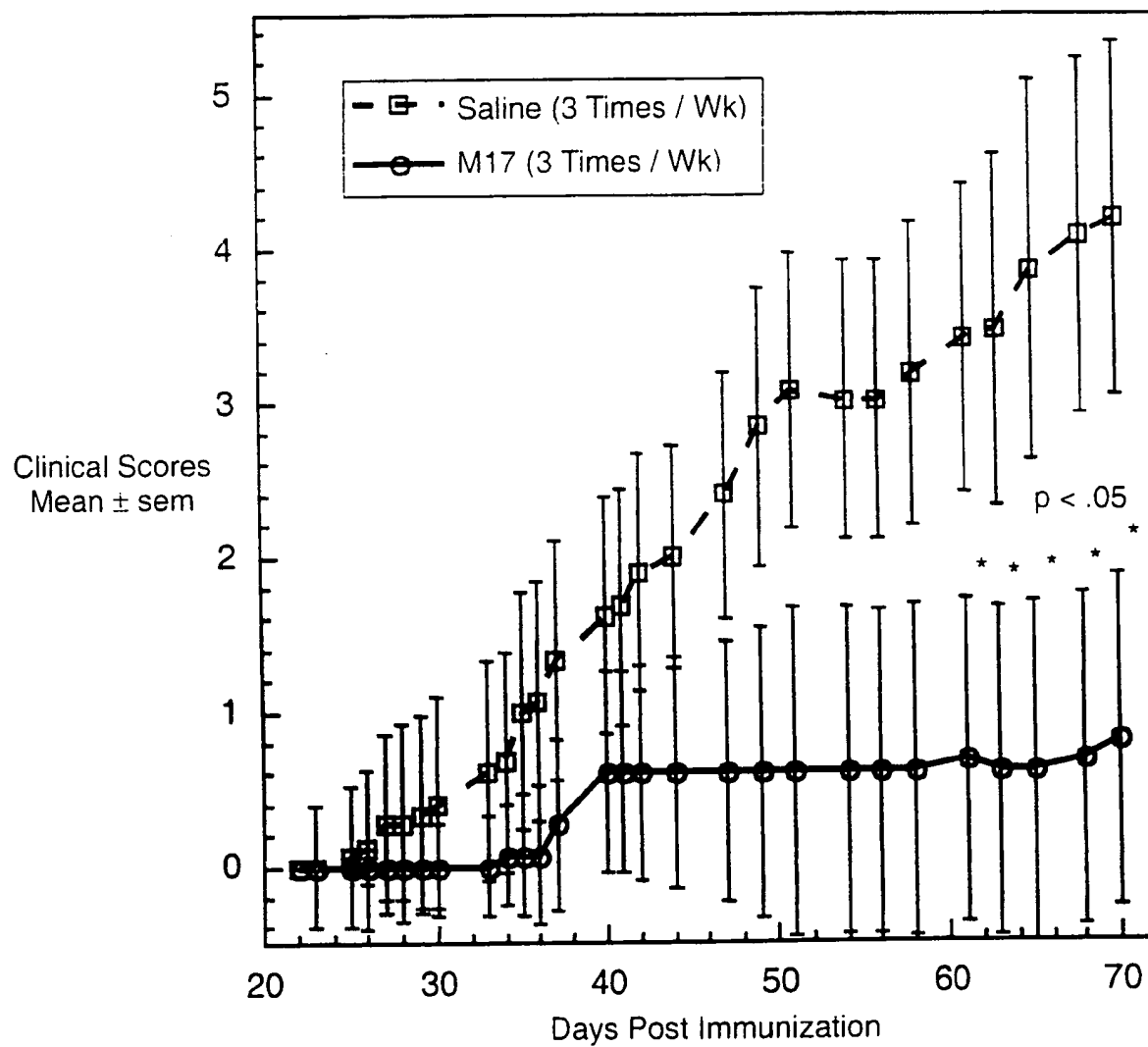
1 / 5

**FIG.\_1**

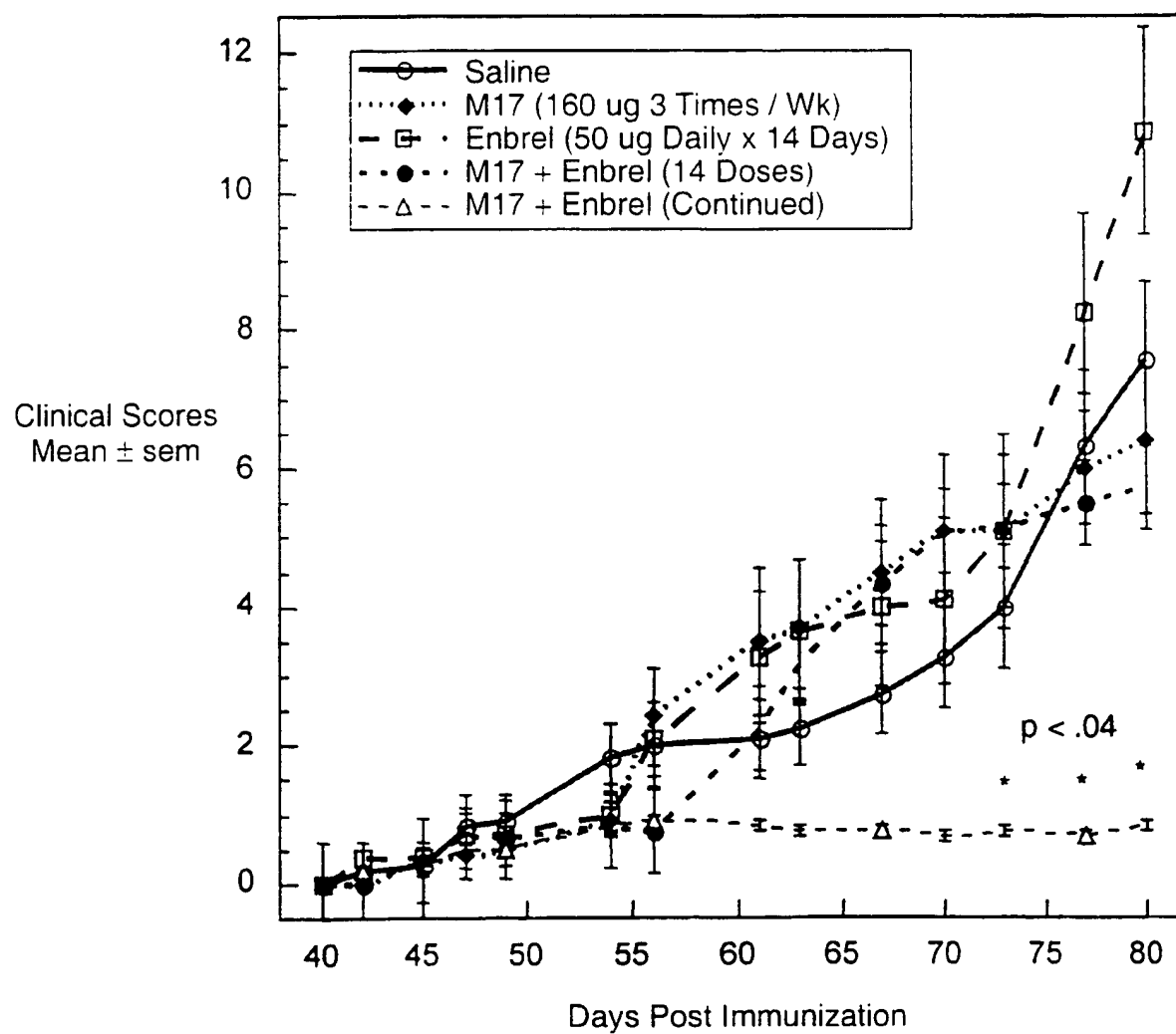
2 / 5

**FIG.\_2**

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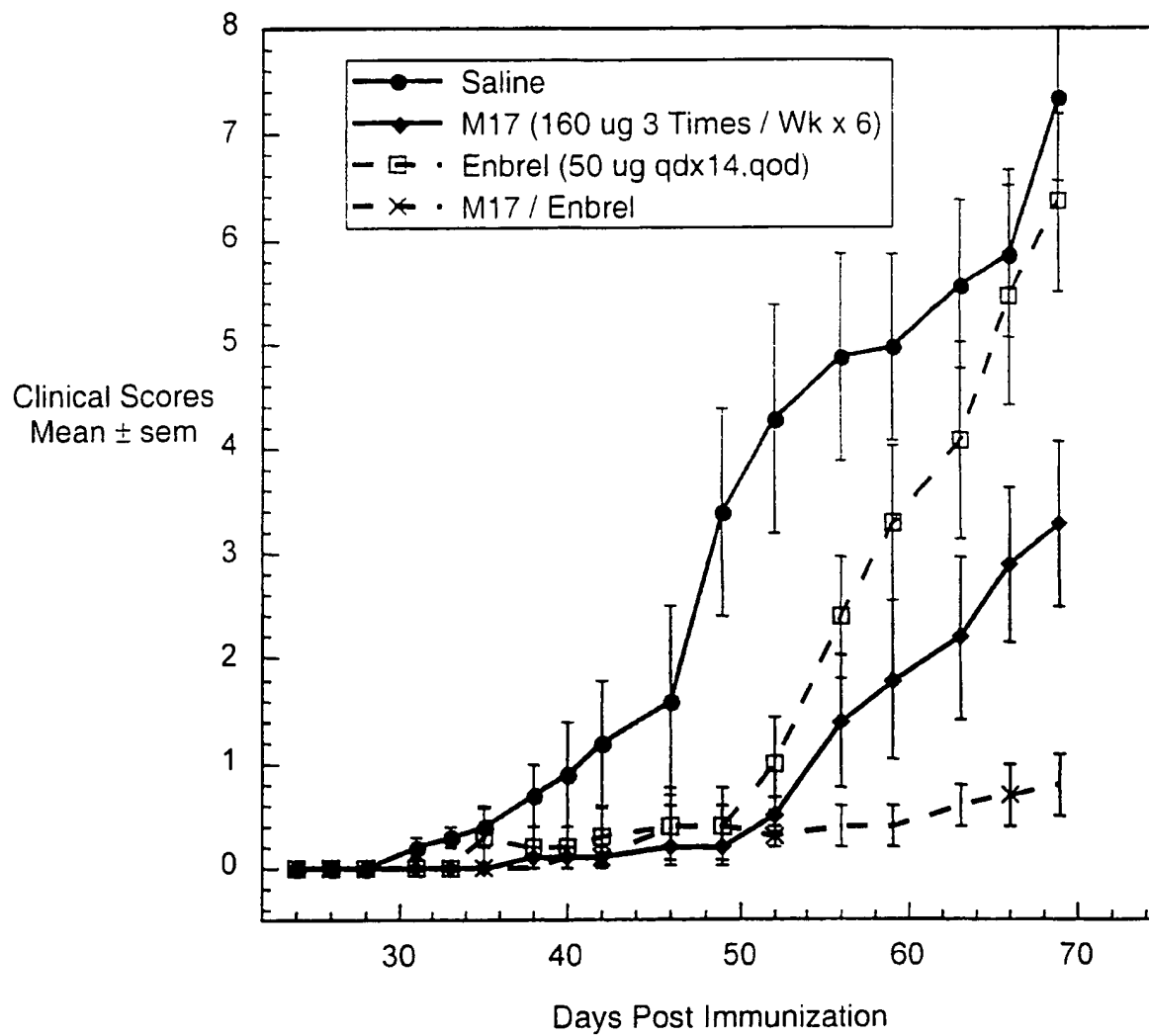
**FIG. 3**

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**FIG.\_4**



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**FIG. 5**

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/33875

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K39/395 A61K38/17 A61P37/06 A61P29/00 //C07K16/28,  
C07K14/715, (A61K39/395, 38:17)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 23761 A (GENENTECH) 4 June 1998 (1998-06-04) page 2, line 31 -page 3, line 7; claims 1-25 page 4, line 14 - line 21 ---	1-16
Y	MORELAND L W ET AL: "LONGTERM TREATMENT OF DMARD FAILING RHEUMATOID ARTHRITIS PATIENTS WITH TNF RECEPTOR P75 FC FUSION PROTEIN (TNF;FC ENBREL)" JOURNAL OF INVESTIGATIVE MEDICINE, AMERICAN FEDERATION FOR CLINICAL RESEARCH,,US, vol. 46, 1998, page 229A XP000972312 ISSN: 1081-5589 abstract --- -/--	1-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

14 May 2001

Date of mailing of the international search report

29/05/2001

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GAO J -X ET AL: "Mac-1 (CD11b/CD18) is the predominant beta-2 (CD18) integrin mediating human neutrophil migration through synovial and dermal fibroblast barriers." IMMUNOLOGY, vol. 88, no. 3, 1996, pages 463-470, XP002167334 ISSN: 0019-2805 the whole document ----	1-16
A	WO 98 22137 A (THE KENNEDY INSTITUTE OF RHEUMATOLOGY) 28 May 1998 (1998-05-28) claims 1-45 ----	1-16
A	LOVELL D I ET AL: "SAFETY AND EFFICACY OF TUMOR NECROSIS FACTOR RECEPTOR P75 FC FUSION PROTEIN (TNFR-FC, ENBREL) IN POLYARTICULAR COURSE JUVENILE RHEUMATOID ARTHRITIS" ARTHRITIS AND RHEUMATISM, LIPPINCOTT, PHILADELPHIA, US, vol. 41, no. 9, SUPPL, 8 November 1998 (1998-11-08), page S130 XP000960458 ISSN: 0004-3591 abstract ----	1-16
T	SPENCER-GREEN G: "ETANERCEPT (ENBREL): UPDATE ON THERAPEUTIC USE" ANNALS OF THE RHEUMATIC DISEASES, BRITISH MEDICAL ASSOCIATION, LONDON, GB, vol. 59, no. SUPPL. 01, November 2000 (2000-11), pages 146-149, XP000965592 ISSN: 0003-4967 abstract -----	1-16

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Information on patent family members

International Application No

PCT/US 00/33875

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